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VOL. XCIX—No. 2

Issued January 1, 1932

BALTIMORE, U. S. A.
1932

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the Act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

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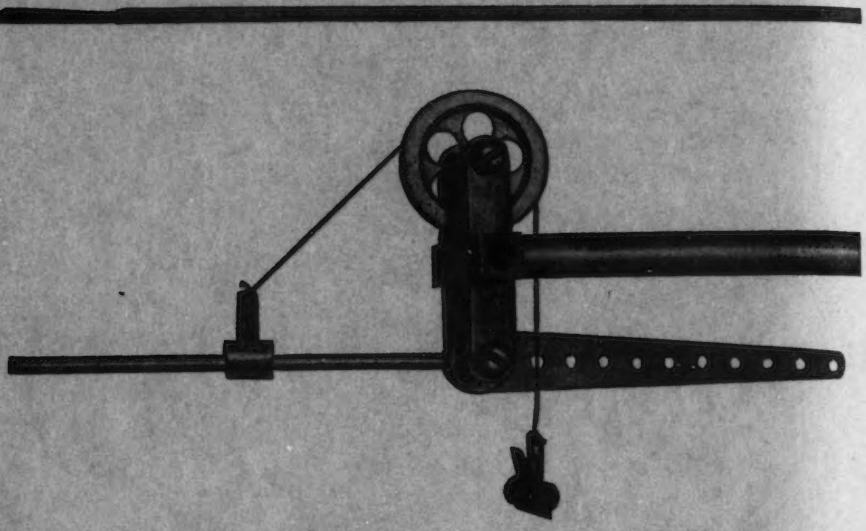

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 99 JANUARY 1, 1932 No. 2

FACTORS IN VENTRICULAR FIBRILLATION[1]

D. R. HOOKER

From the Department of Physiological Hygiene of the Johns Hopkins University

Received for publication May 29, 1931

A year ago the writer reported experiments (1930) which showed that an increase of sodium bicarbonate, irrespective of its buffer action, or an increase of calcium chloride in the perfusate prepared for use in the isolated dog heart, conduced to the establishment of ventricular fibrillation. If the two salts were both increased in the same solution the effect was even more pronounced, so that a sudden transfer from normal Locke solution to such a perfusate would sometimes throw the ventricles into fibrillation without the interaction of other factors. These results were regarded as supporting an hypothesis originally advanced by Gotch, that ventricular fibrillation, whether produced by electrical stimulation or otherwise, may be due to a dissociation of some protein-salt molecule, as conceived by J. Loeb (1906), such that an abnormal chemical state exists in or surrounds the muscle fibers with a consequent change in their behavior.

Further support of the concept that chemical factors may play an hitherto unsuspected rôle in ventricular fibrillation, specifically in connection with the sodium chloride content of the perfusate, is to be found in the observations reported in this paper but it is desired first briefly to present some experiments, negative in outcome, which were designed to locate a fibrillary center, as was implied in Kronecker's puncture, or some region in the ventricular mass more susceptible to fibrillation than the remainder of the cardiac structure. Although no one has reported confirmation of Kronecker's observation (1884) that a needle thrust into the anterior interventricular sulcus at a particular point, i.e., at the lower end of the upper third of this sulcus, will produce ventricular fibrillation, our present knowledge of cardiac physiology, particularly as regards the sig-

[1] Published with the approval of the Physiological Committee of the Conference on Electric Shock.

nificance of the nodal "centers" and conducting system, is suggestive of the possibility that the phenomenon might have some selective locus in spite of the fact that fibrillation spreads from an electrically irritated point to the whole ventricular mass with such promptitude as to appear instantaneous. Early experiments appeared to support such a view but the subsequent more careful approach to the problem was completely negative in outcome.

No region particularly susceptible to fibrillation or in which fibrillation is conspicuously persistent can be located. If the ventricles of the perfused dog heart be thrown into fibrillation by electrical stimulation, pieces of considerable size can be cut away without seriously disturbing the nutrition of the residual mass provided hemostats are used to occlude the severed vessels. Under such circumstances the perfused heart will continue to fibrillate while the several pieces will usually cease to fibrillate promptly. Sometimes they exhibit independent beats but more often they are quiescent except for single beats in response to mechanical or brief electrical stimuli. During prolonged tetanic stimulation they will remain in fibrillation only to fall back almost at once into a state of quiescence or of independent beats. These statements apply particularly to pieces taken from the apex (weighing up to 30 per cent of the whole ventricular mass) and from the right ventricular wall (weighing up to 20 per cent of the whole ventricular mass). While some evidence was collected which seemed to show that pieces from the basal regions of the septum and left ventricle continued to remain in fibrillation longer after being cut away the results were not consistent. Consequently it is fair to say that pieces of the size indicated, severed from any part of the fibrillating ventricles, will promptly cease to fibrillate while the remainder continue in fibrillation.

Because of the possibility that these results might be due to the nutritional state of the severed piece recourse was had to separate perfusion of the area which was to be cut away. This called for separate cannulation and perfusion of the different coronary branches in different experiments. When the operative manoeuvers, performed under potassium inhibition, were successfully completed, the experimental area and the rest of the heart were being supplied with the same perfusate under approximately identical conditions. After fibrillation was established the experimental area of muscle tissue was cut away and transferred to a suitable support without interruption of its circulation. Obviously it was not possible by this procedure to select many places on the left ventricular wall but enough of them could be found for correlation with the results of the simpler procedure.

The results obtained by both methods were in essential agreement. The separately perfused piece stopped fibrillating promptly when severed from

the remainder of the heart. There was this difference, however: the severed and perfused piece invariably began to beat spontaneously in a short time and continued to beat indefinitely while the heart mass remained in fibrillation. The following is a typical protocol:

November 5, 1930. Dog, female, 12 pounds. Morphia-ether. Thorax opened, heart isolated and perfused with Locke at 60 mm. Hg and 35°C. Inhibited by injecting 3 cc. 3 per cent KCl into perfusion cannula. Perfusion stopped. Cannulated posterior branch of circumflex (supplying mid-basal region of left ventricle). Perfusion started; heart beating well. Fibrillated ventricle with 5 seconds' tetanic stimulation.

Cut isolated piece away (weight 7 gm. = ± 20 per cent ventricular mass). Piece stopped fibrillating and began to beat spontaneously within 5 seconds.

Six minutes after excision stimulated piece with tetanic stimulation for 5 seconds without lasting effect. Repeated stimulation for 10 seconds, after which it remained in fibrillation for 7 seconds and then reverted to spontaneous beats.

Discarded this piece and cannulated anterior branch (supplying septal region of anterior part of left ventricle). Occupied about 20 minutes during which perfusion was maintained and heart continued in fibrillation.

Cut second isolated piece away (weight 16 gm. = ±45 per cent ventricular mass). It continued in fibrillation for 5.5 minutes when it was inhibited by injection of 0.5 cc. 3 per cent KCl. When it recovered from inhibition it began to beat spontaneously.

Stimulated 5 seconds without lasting effect; stimulated 10 seconds, after which fibrillation continued for 20 seconds, when spontaneous rhythmic beats again appeared. Throughout the whole period occupied by these manipulations the residual heart mass remained in fibrillation.

This experiment, aside from pointing the statements made above, exhibits a phenomenon frequently observed and for which no explanation can be offered, i.e., the greater persistency of fibrillation (as seen in the second isolated piece) the more thoroughly it has been established. This characteristic may be found in any part or in the whole heart and, while it seems to add to the elusiveness of the basic problem of fibrillation, it suggests again that the fundamental issue is related to the inherent metabolic state of the individual muscle fibers.

It was at first thought that the sudden fall in temperature might be a contributing factor in these results but when the severed pieces of tissue were quickly transferred to a bath of warm Locke solution they ceased to fibrillate as promptly as when exposed to cooling air. Further evidence that temperature is not a factor was found in experiments in which the fibrillating heart was brought to a complete standstill for two minutes with a cold solution (13°C. at cannula, 17.5°C. at outflow). When a perfusate of normal temperature (35.5°C.) replaced the cold the ventricles at once returned to vigorous fibrillation.

The foregoing observations fail to lend any support to the concept of a fibrillary center and seem rather to indicate that the disturbance may

originate anywhere in the ventricular mass. Further support of this negative evidence is found in the results which fail to show a greater tendency for the fibrillation to persist in one part of the heart than in another.

Part which sodium chloride may play in fibrillation. Increasing the amount of $CaCl_2$ or of $NaHCO_3$ or both in the perfusate conduces to ventricular fibrillation (l.c.). It is now possible to show that an upset in the NaCl balance, irrespective of the osmotic pressure, exerts a profound influence in the same respect upon the behavior of the isolated heart. If the amount of NaCl is increased in a Locke solution the rate of a perfused heart is slowed and this slowing is accompanied by cardiac relaxation. Furthermore, such a heart is less irritable to electrical stimulation. If, on the other hand, the amount of NaCl is decreased, the rate of the heart is increased and if the decrease in the NaCl content is sufficient the ventricles will fall into fibrillation.

This NaCl effect is of interest primarily because it evidences another chemical factor in ventricular fibrillation and incidentally because of its possible value in the development of remedial measures to overcome fibrillation. As will be seen the upset in NaCl balance must be extreme in order to produce its effect but this might be anticipated due to the relative preponderance of NaCl in the salt content of a "normal" perfusate.

Effect of increasing the NaCl content of a perfusate. When transition is made from a normal Locke solution to an otherwise similar one in which the NaCl is considerably increased (from 0.9 per cent to 1.58 per cent) the heart is slowed and apparently dilated. Increased osmotic pressure is not a factor in this reaction since if the increased NaCl is roughly compensated by a decrease in CaCl, KCl and dextrose the same type of response is obtained. A heart in this state requires a stronger electrical stimulation to be thrown into fibrillation and a heart transferred to the high NaCl perfusate, after being fibrillated while perfused with normal Locke, appreciably changes the type of fibrillation exhibited in that the twitching becomes more discrete and sluggish. The latter observation led to an unsuccessful attempt to recover a fibrillating heart by coronary injection of 3 per cent NaCl. While this attempt was a failure the idea is perhaps worth further study since the disturbance did seem to quiet down for a brief moment.

A protocol setting forth some of these effects follows:

May 4, 1931. Dog under morphia and ether prepared for perfusion of heart. Perfusates used:

I. Normal Locke solution.

II. Locke solution made up with 1.58 per cent NaCl and approximately compensated osmotically by a decrease in $CaCl_2$, KCl and dextrose.

Perfusion started with solution I. Fibrillation established with secondary coil at 11. KCl inhibition with recovery.

2 m. 45 s. Changed to solution II.
10 m. 30 s. No fibrillation with secondary coil at 7.
10 m. 45 s. Changed to solution I.
13 m. 30 s. Fibrillation established with secondary coil at 11. KCl inhibition with recovery.
15 m. 30 s. Changed to solution II.
20 m. 20 s. No fibrillation with secondary coil at 7.
20 m. 45 s. Changed to solution I.
23 m. 45 s. Fibrillation established with secondary coil at + 13. Experiment discontinued.

The lessened irritability or decreased tendency to go into fibrillation shown by this heart when perfused with a solution strong in NaCl was not due to the decrease in the other constituents of Locke solution. This was readily shown by using the normal amounts of $CaCl_2$, KCl and dextrose with the increased amount of NaCl (i.e., 1.58 per cent) and comparing the effect of such a solution with normal Locke. The results were essentially similar to those given above and indicate that we are dealing with a NaCl effect.

Effect of decreasing the NaCl content of a perfusate. When transition is made from a normal Locke solution to an otherwise similar one in which the NaCl is considerably decreased (from 0.9 per cent to 0.09 or 0.23 per cent) the heart rate is sharply accelerated and ventricular fibrillation develops in a short time without the intervention of other factors. This result is the exact opposite of that obtained when using an increased amount of NaCl.

Here again the disturbance is not dependent upon change of osmotic pressure as such because if the decrease in NaCl content of the perfusate is osmotically compensated by balanced addition of $CaCl_2$, KCl and dextrose a similar result follows.

If a heart is isolated on Locke solution in the usual manner until a constant rhythm is established and then transfer is made to an identical solution except in being low in NaCl (i.e., 0.09 per cent) fibrillation will develop in about one minute. If, on the other hand, perfusion be begun on the Locke low in NaCl fibrillation will develop much more quickly, say in twenty seconds. The quicker reaction in the latter case is of interest. It is undoubtedly due to the sudden transition from circulatory blood to an inadequate perfusate. The fact that when the transition is made from circulating blood to Locke solution and then to the inadequate perfusate fibrillation develops with appreciably less suddenness, agrees with the accumulating evidence (Ort, Power and Markowitz) that Locke solution is not to be regarded as an ideal perfusate for the heart.

These statements are based upon the use of 0.09 per cent NaCl but they are equally applicable when 0.23 per cent NaCl is used. When, however, the use of 0.45 per cent NaCl is considered the result is different. Here the

rate of the heart beats is markedly accelerated but fibrillation is not developed.

The several experiments considered in the foregoing paragraphs show a definitely graded response of the isolated dog heart to varying concentrations of NaCl in the perfusate. High concentrations (1.58 per cent) bring about a slow beating and dilated heart; low concentrations (0.23 per cent) produce ventricular fibrillation. Passing from the higher to the lower concentrations the irritability of the tissue is increased, the "tone" of the muscle fibres presumably rises and the rate of contractions is accelerated until the accelerated rhythm finally passes into the disordered state of fibrillation.

Bouckaert and Belehradek (1927) carried out a similar study on perfused frog skeletal muscle with quite different results. They found that maximal contraction of the gastrocnemius, subjected to uniform stimulation, is obtained when the NaCl content of the perfusate is at 0.2 per cent. Their published curve shows the first contractions at 0.075 per cent of NaCl, rises to a maximum at 0.2 per cent and falls off again to zero at 0.65 per cent. Obviously the only explanation of these divergent results to be offered at the present time is to be found in the reaction of different tissues which again raises the question of what is an adequate perfusate.

SUMMARY

No region of the isolated dog heart is particularly susceptible to fibrillation and no region can be found in which fibrillation is conspicuously persistent when once established.

Variation in the sodium chloride content of Locke perfusate, irrespective of osmotic pressure and irrespective of the other saline constituents, exerts a profound influence upon the behavior of the isolated dog heart. If the content of sodium chloride is high (1.58 per cent) the heart appears to be relaxed, beats slowly and is less irritable to electrical stimulation; if the content is below normal (i.e., 0.45 per cent) the heart rate is greatly increased, while if the content is reduced still further (i.e., 0.23 per cent) the ventricles are thrown into fibrillation.

Attention is called to experiments on frog skeletal muscle in which variation in the sodium chloride content of the perfusate exerts a very different effect than that here reported for the dog heart.

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STUDIES ON SUPRARENAL INSUFFICIENCY

IX. Vascular Responses to Histamine in Normal and in Suprarenalectomized Rats

LELAND C. WYMAN and CAROLINE TUM SUDEN

From the Physiological Laboratory of Boston University School of Medicine and the Evans Memorial, No. 284

Received for publication June 27, 1931

Normal albino rats have a great natural resistance to histamine poisoning, the minimal lethal dose being around 100 mgm. per 100 grams of body weight. This natural resistance is much reduced in suprarenal insufficiency, suprarenalectomized rats being about 20 times more susceptible to histamine than normal rats (Voegtlin and Dyer, 1925; Crivellari, 1927; Scott, 1928; Marmorston-Gottesman and Gottesman, 1928; Wyman, 1928). In a previous paper of this series it was reported that this increased susceptibility is largely due to the lack of medullary tissue, rather than to lack of cortical tissue (Wyman, 1928). This conclusion was based on the fact that suprarenalectomized rats possessing gross accessory or transplanted cortical tissue are as susceptible to histamine as rats suffering from cortical insufficiency, and that intraperitoneal injections of small amounts of adrenalin afford partial or complete protection against fatal histamine poisoning in the operated rats. It has been our opinion that the administration of a poisonous dose of histamine constitutes an emergency which is met by the suprarenal medulla in the usual way, that is, by the secretion of an extra amount of adrenin, which acts as a physiologically antagonistic agent to combat the effects of the histamine.

In 1929, Perla and Marmorston-Gottesman reported that they were not able to protect suprarenalectomized rats against lethal doses of histamine by single subcutaneous injections of adrenalin, but that they were able to protect them in 50 per cent of cases by subcutaneous injections of adrenalin twice daily during seven days following suprarenalectomy. The last injection, however, had to be given two hours prior to the injection of histamine. They also claimed that the height of susceptibility to histamine occurs at the end of the first week following operation, taking at least five days to develop. They concluded, therefore, that the protective action of adrenalin is dependent not so much on its immediate pharmacologically antagonistic action as on its continuous effect on a protective systemic mechanism, maintaining the nutrition or tone of perhaps the

autonomic nervous system. In the hope of throwing some light on these and allied problems, an investigation of the vascular responses of rats in various conditions to intravenous injections of histamine was begun.

METHODS. A mixed stock of colored varieties of the albino rat was used. Details regarding the general technique of studying suprarenal insufficiency in rats have been described in previous papers in this series. For intravenous injection, ergamine acid phosphate (Burroughs, Wellcome & Co.) was dissolved in sterile physiological saline solution so that any one injection would be only a few tenths, or less, of one cubic centimeter. For subcutaneous injection, adrenalin chloride solution (Parke, Davis & Co.) was freshly diluted in sterile physiological saline solution to make a 1:10,000 dilution. Carotid blood pressures were measured by the mercury manometer method, using the cannulas designed by Durant (1927), manufactured from hypodermic needles. The femoral vein was cannulated for intravenous injections. Heparin (Hynson, Westcott & Dunning), 10 mgm. per rat, was injected intravenously to prevent clotting, and physiological saline solution was used in the manometer connections to do away with the bad effects of drawing an anticoagulant solution into the carotid artery during an extensive fall in blood pressure. Urethane anesthesia, 0.15 gram per 100 grams body weight, was used throughout. Complete autopsies were performed on all rats.

Development of susceptibility. In order to find how soon increased susceptibility to histamine may be demonstrated after suprarenalectomy, a series of 47 rats was prepared in various ways, and intraperitoneal injections of 10 mgm. of histamine per 100 grams body weight were given on the day of operation, or on the first, second or third days following operation. This dose of histamine is lethal for most suprarenalectomized rats, but is harmless for normal rats. Control injections of normal saline solution were also used. The results are given in table 1. All of the operated controls survived following the injection of histamine. Of 31 rats with the suprarenals eliminated by one means or another 26 died following the histamine injection. The five which survived had had the suprarenals ligated, but not removed, allowing opportunity for incomplete occlusion of the suprarenal vessels. Although experiments of this type done on the day of operation or shortly following may be justly criticized, the behaviour of the controls and the increased susceptibility following simple ligation of the glands, indicates that the increased susceptibility to histamine in suprarenalectomized rats follows immediately the removal of the influence of the suprarenal medulla from the circulation.

Direct observation of blood vessels. Direct microscopic observation of the reactions of blood vessels during histamine administration was accomplished by means of the abdominal window method (Mendenhall, 1931). A rectangular celluloid window, about 3.5 by 4.5 cm., was inserted between

the skin and the abdominal muscles, covering an opening through the muscles into the cavity. In cases where direct application of solutions to the surface of the intestines was to be made, a window with a small hole which could be closed by a cork was used. Such a window soon becomes cemented to the edges of the skin incision by dried exudate; thus the intestines are well protected and the blood vessels appear to maintain a normal tonus. This method is far preferable to one which would involve handling or exposure of the intestines. Direct observation of blood vessels on the surface of the cecum, on the ileum, or in the mesentery, was made by reflected light with a binocular microscope, while intravenous injections of histamine were being given, or when solutions were directly applied to the surface of the intestines by means of a pipette.

TABLE 1

OPERATION	INTRAPERITONEAL INJECTIONS OF 10 MGM. OF HISTAMINE PER 100 GRAMS BODY WEIGHT GIVEN ON—								CONTROL INJECTION OF SALINE SOLUTION GIVEN ON—	
	Day of operation		1 day after		2 days after		3 days after		Day of operation	
	Died	Survived	Died	Survived	Died	Survived	Died	Survived	Died	Survived
Blank operated controls	0	3	0	1			0	3		
Singly suprarenalectomized controls	0	4			0	1				
Suprarenalectomized without ligation of suprarenals	5	0					2	0		
Suprarenalectomized after ligation of suprarenals	2	0	4	0	2	0			0	1
Suprarenals ligated and left in situ	6	5	5	0					0	3

An interesting characteristic of the resting arteries was the occurrence of alternate relaxations and constrictions similar to the "oscillations in calibre" seen by Grant (1930) in the arteries of the rabbit's ear following pricking the skin near an artery, or the application of histamine or adrenalin. These oscillations were present in the arteries on the surface of the intestine in nine of the eleven animals examined. Simultaneous observations made by two persons through a double eyepiece showed that these oscillations were synchronous throughout a single arterial branch, rather than being waves of constriction, but that they were not synchronous in different arterial branches. The rates of oscillation were quite similar in different animals. The relaxation periods averaged 7.4 seconds and the constriction periods averaged 5.77 seconds, for 75 measure-

ments. When marked dilatation was produced by histamine or marked constriction by adrenalin the oscillations disappeared, returning as the effects of the drugs wore off. Inasmuch as the amplitude and rate of oscillation were changed by these substances, such changes were useful in detecting effects produced by very small doses which did not cause alterations in calibre large enough to be seen. No oscillations in calibre were seen in the vessels of the ear. Since Grant observed them only after injury or the application of histamine the question arises, does their appearance in the arteries of the intestines but not in those of the ear, indicate the liberation of some substance in the intestine during the slight amount of handling involved in inserting the window? Further work is indicated.

Sufficient doses of histamine produced dilatation of all vessels observed, even of the larger arteries in the mesenteries. No signs of a constrictor action were seen. Direct application of solutions of histamine, 1:1000 or 1:10,000, to the surface of the gut caused dilatation of arteries, veins and capillaries. The effect first appeared in the arteries in about 20 seconds, and later in the veins and capillaries, lasting from two to five minutes. The smallest intravenous dose of histamine which produced a detectable effect on the arteries was 0.001 mgm. per 100 grams body weight. The time of onset of the dilatation in the arteries was about the same for all intravenous doses tried, i.e., about 10 seconds. The duration of dilatation increased with the dose, corresponding roughly with the duration of the depressor action for corresponding doses seen in blood pressure experiments. Doses of 0.5 mgm. per 100 grams, which produce permanent falls in blood pressure in normal rats, produced dilatations from which there was incomplete recovery. Doses of from 0.001 mgm. to 0.02 mgm. per 100 grams dilated only arteries, and doses of from 0.05 to 0.5 mgm. per 100 grams dilated arteries, veins and capillaries in the order given. Control injections of normal saline solution up to amounts considerably greater than those of the histamine injections produced no visible effects on the blood vessels.

Direct microscopic observation of the blood vessels of the ear by transmitted light had the advantage of allowing measurement of their diameters by means of a micrometer ocular, a procedure which is hampered by the respiratory movements in the case of intestinal vessels. In general the results confirmed the observations on intestinal areas. No oscillations in calibre were seen in the arteries. The time of onset of the dilatation of arteries following intravenous injections of histamine was somewhat longer than in the case of the intestinal vessels, being from 15 to 20 seconds. The duration of dilatation was about the same. The larger arteries showed dilatation but the main trunk of the central vein was not visibly affected. The smaller terminal branches of the veins, near the edge of the ear,

dilated, however, following histamine injections. Capillaries could not be seen distinctly but flushing of the field indicated capillary dilatation. Recovery from the dilatation was marked by a constriction of the arteries beyond the normal state of tonus, a very slight constriction of the larger veins, and a paling of the field more than normal. The latter two conditions may have been passive. A slow return to the normal state of tonus of the arteries and color of the field then ensued. The reason for this overcompensation in recovery was not determined.

In 1926 Burn and Dale summarized the effects of histamine in various animals as follows. On the arterial side of the circulation the action consists of a constrictor effect on the more central part of the vascular tree, changing to a dilator effect on the more peripheral branches. In the dog and probably in the monkey dilatation occurs before the smallest macroscopic arteries are reached. In the cat it occurs more peripherally, so that the dilator effect is mainly if not entirely exercised on the capillaries. In the rodents the dilator effect is pushed so far to the periphery as to escape detection and possibly does not exist at all. In the following year Feldberg (1927) showed, however, that in the rabbit histamine has a dual action, a constrictor effect on the visible arterial branches, and a more peripheral dilator effect, spreading on to the capillaries, thus bringing the rabbit into line with the cat. The observations described above bring the action of histamine on the rats vessels into line with what was previously known of its action on those of the dog. This is interesting in view of the fact that the type of anaphylactic shock seen in the rat resembles that in the dog.

Vascular responses to histamine. An attempt was made to demonstrate differences in the vascular reactions to intravenous injections of histamine solutions in rats of various types. Blood pressure experiments were done using normal rats, blank operated controls, suprarenalectomized rats during the second or third week following operation before marked symptoms of insufficiency had appeared, and suprarenalectomized rats having autoplastic transplants of cortical tissue or gross accessory cortical tissue but no demonstrable medullary tissue.

The blood pressures of these types of rats are given in table 2. Of the normal rats only four cases had a blood pressure above 128 mm. Hg, and three cases a pressure below 94 mm., hence the normal range is probably between these limits. The figures agree well with those of Durant (1927), although he found the average for 18 males to be 123.5 mm. Hg, a figure somewhat higher than ours. The results with the blank operated rats indicate that surgical trauma per se does not lower the blood pressure for long. Of the suprarenalectomized group having transplanted or accessory cortical tissue only three had a pressure below 90 mm. Hg. The figures here are similar to those in the case of normal rats. The blood pressures

of suprarenalectomized rats without cortical tissue, however, were distinctly lower. Eliminating six of this group which had severe symptoms of suprarenal insufficiency with pressures from 24 to 48 mm. Hg, and two cases with pressures of 100 and 110 mm. Hg, the range becomes 54 to 86 mm. Hg, and the average 72.6 mm. Hg. This agrees with Durant's figures (1928) for nine rats, measured from 6 hours to 22 days after suprarenalectomy. The above data indicate that the low blood pressure seen in suprarenal insufficiency in rats is concurrent with cortical rather than with medullary defect.

TABLE 2

Blood pressures in millimeters Hg

	NUMBER OF MALES	NUMBER OF FEMALES	DAYS AFTER OPERATION	WEIGHT	WEIGHT LOSS SINCE OPERATION	BLOOD PRESSURE RANGE	BLOOD PRESSURE AVERAGE	DURANT, 1927, 1928		
								Number of cases	Blood pressure range	Blood pressure average
				grams		*mm.* Hg				
Normal rats.....	31	0		250 to 350		85 to 142	110.2	41	92 to 150	119
Blank operated control rats.....	3	1	6 to 8	213 to 280	Gain	106 to 130	121	10	65 to 135	111
Suprarenalectomized rats with transplanted (9) or accessory (3) cortex.....	6	6	Transplanted, 27 to 51; accessories, 9 or 300	190 to 325	Gain	82 to 142	108			
Suprarenalectomized rats.....	22	2	7 to 20	190 to 300	3 to 65	24 to 110	67.4	9	52 to 95	77

Histamine, injected into the femoral vein, was found to produce a depressor action with all effective doses tried. No evidence of a pressor or constrictor action was ever seen. The minimal dose which would produce a detectable fall of pressure was about 0.0001 mgm. (fig. 1). To test the responses to small doses three normal rats, three transplanted rats and two found to have gross accessory cortical masses ten months after operation, and three suprarenalectomized rats 14 to 18 days after operation were used. All were males, weighing around 300 grams, and the suprarenalectomized rats were in good condition. Little difference could be seen among these

types with respect to their reactions to small doses of histamine which produced small, transient depressor responses, i.e., doses of from 0.0001 mgm. to 0.001 mgm. per 100 grams body weight. Apparently the smaller doses, i.e., 0.0001 mgm. or 0.0002 mgm. absolute amount, or 0.0001 mgm. and 0.0002 mgm. per 100 grams, produced slightly greater depressor responses in the suprarenalectomized, transplanted and accessory rats than in the normal rats. The results were so irregular, however, that the data could not be used as standards for other types of experiment. Somewhat larger doses, up to 0.001 mgm. per 100 grams, produced similar depressor responses in all types of rat.

The depressor responses increased in magnitude with increasing dosage up to between 0.004 mgm. and 0.01 mgm. per 100 grams (fig. 1), but beyond this, increasing the dosage resulted chiefly in increase in the time of recovery. Apparently the maximum dose for depressor effect lies between the limits indicated. The injections of histamine solutions were carefully controlled with respect to volume, speed of injection, and temperature, by injections of normal saline solution. No effect on the blood pressure was noted (fig. 1).

Although the results following small doses of histamine were not adequate to distinguish differences in the vascular responses of control and suprarenalectomized rats, in the case of larger doses a distinct difference was found. This was done by determining the minimal dose of histamine necessary to produce a permanent fall of blood pressure, that is, a failure to recover to the previous level of pressure during the subsequent course of the experiment (from one to three hours). For normal and blank operated rats this dose is from 0.3 to 0.5 mgm. per 100 grams body weight (24 cases), and for suprarenalectomized rats, or for suprarenalectomized rats having transplanted or gross accessory cortical tissue, it is from 0.05 to 0.1 mgm. per 100 grams body weight (suprarenalectomized 16 cases, transplants 7 cases, accessories 4 cases) (see figs. 2, 3, 4 and 5). The difference is very clean cut and quite suitable as a criterion for further work.

In view of the suggestion of Perla and Marmorston-Gottesman that daily injections of adrenalin following suprarenalectomy maintain the tone of some protective mechanism, probably vascular, their technique with regard to adrenalin injections has been carefully followed in a series of 12 rats, 10 males and 2 females, and blood pressure experiments have been done immediately following this treatment. Subcutaneous injections of adrenalin, 0.02 mgm. per 100 grams body weight, were given twice daily during seven or eight days following suprarenalectomy, the last injection being given two hours prior to the injection of histamine in the blood pressure experiment. The treatment with adrenalin did not materially affect the vascular responses. With one exception, the twelve rats showed

responses similar to those of untreated rats having no demonstrable chromaffin tissue, i.e., doses of 0.1 mgm. of histamine per 100 grams body weight produced permanent falls in blood pressure. Two of this series had gained 4 grams apiece since operation and were found at autopsy to have gross accessory cortical tissue. The others had lost from 23 to 49 grams since operation, which is not unusual following suprarenalectomy. In some cases the initial blood pressure was higher and the vascular reactions to histamine were not so marked as in the case of suprarenalectomized rats with no previous treatment. Eliminating the two having accessory cortical tissue, the initial blood pressure range was from 74 to 124 mm. Hg, averaging 90.8 mm. Only four cases, however, had initial pressures above 90 mm. Hg. which is about the minimum for normal rats and the maximum for suprarenalectomized rats, and of these only one had a pressure above 96 mm. (124 mm. Hg).

It was felt that the last subcutaneous dose of adrenalin, which must be given two hours before the injection of histamine, according to the technique of Perla and Marmorston-Gottesman, might be exerting a pharmacological effect which might account for these slight differences from the results in the untreated suprarenalectomized series. It has been adequately demonstrated that adrenalin so administered is absorbed over a long period and is not entirely destroyed for some time in such a location. This was confirmed for the rat, using one normal and five suprarenalectomized animals seven days after operation, by giving a subcutaneous dose

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Fig. 1. Experiment 11. Normal male rat, 292 grams. In this, and in subsequent tracings, the time record shows five second intervals and is at zero blood pressure level. Injections of histamine solution at *H*. A, 0.0001 mgm. histamine (0.01 cc. sol.); B, 0.0002 mgm. histamine (0.02 cc.); C, 0.0001 mgm. histamine per 100 grams body weight (0.029 cc.); D, 0.0002 mgm. histamine per 100 grams (0.058 cc.); E, 0.0004 mgm. per 100 grams (0.117 cc.); F, 0.001 mgm. per 100 grams (0.29 cc.). Control injections of normal saline solution at *S*. *G*., 0.02 cc. saline solution; *H*, 0.03 cc. saline solution; *I*, 0.1 cc. saline solution; *J*, 0.2 cc. saline solution.

Fig. 2. Experiment 35. Normal male rat; A, 0.05 mgm. histamine per 100 grams body weight (recovery); B, 0.1 mgm. histamine per 100 grams (recovery); C, 0.3 mgm. histamine per 100 grams (permanent fall of blood pressure).

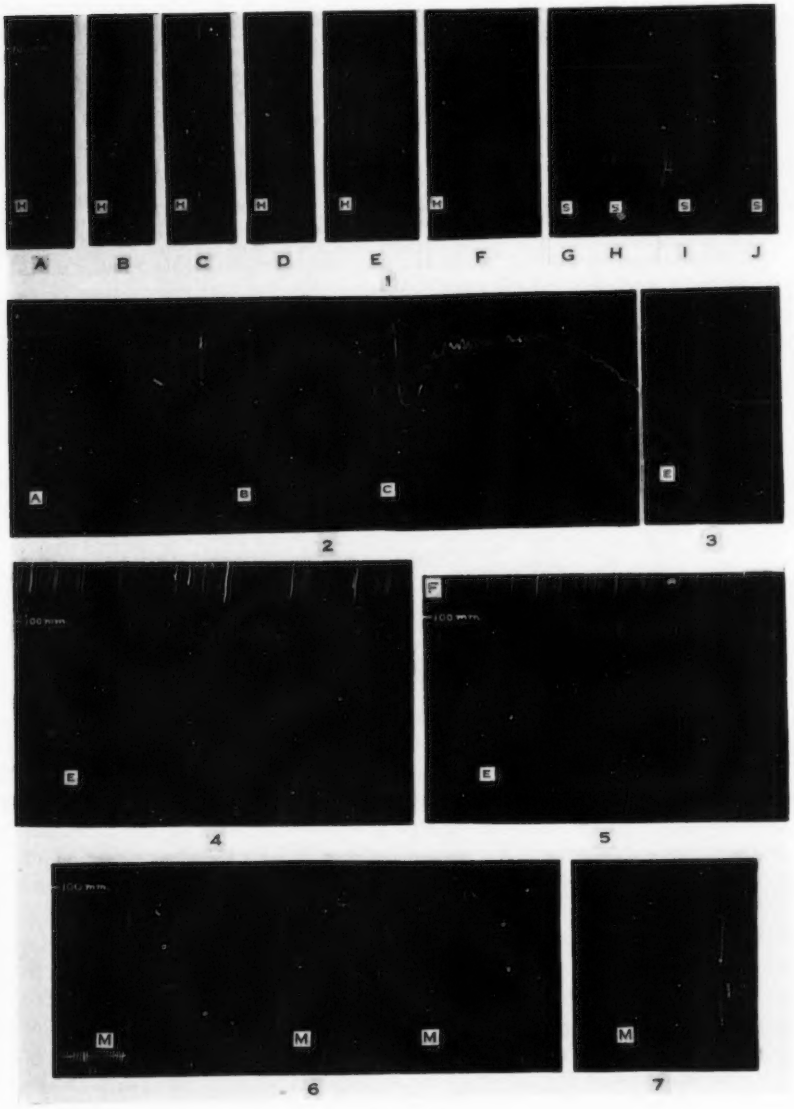
Fig. 3. Experiment 45. Normal male rat, 275 grams. *E*, 0.5 mgm. histamine per 100 grams (permanent fall).

Fig. 4. Experiment 59. Male rat, 240 grams, having transplanted cortical tissue but no demonstrable chromaffin tssue; *E*, 0.1 mgm. histamine per 100 grams (permanent fall).

Fig. 5. Experiment 54. Male rat, 250 grams, 11 days after suprarenalectomy; *E*, 0.1 mgm. histamine per 100 grams (permanent fall).

Fig. 6. Experiment 87. Normal female rat, 240 grams.

Fig. 7. Experiment 90. Male rat, 292 grams, 7 days after suprarenalectomy. Subcutaneous injection of 0.02 mgm. of adrenalin per 100 grams body weight in each case. *M*, *M*, *M*, *M*, massage of site of injection 2 hours later.



Figs. 1–7

of 0.02 mgm. of adrenalin per 100 grams body weight, either before or at the beginning of a blood pressure determination, and gently massaging the site of injection at widely spaced intervals. Although the results were not constant a definite rise in the level of blood pressure was frequently seen upon massaging the site of injection from one to three hours later (figs. 6 and 7). Therefore, twelve experiments were done in which the time relations with respect to operation, injection of histamine, etc., were the same as in the daily treated series described above, with the exception that the rats did not receive any adrenalin until two hours before the injection of histamine in the blood pressure experiment, when a single subcutaneous dose of 0.02 mgm. of adrenalin per 100 grams was given. The results were exactly similar to those obtained in the series which had daily treatments with adrenalin. With two exceptions permanent falls in blood pressure occurred following the injection of 0.1 mgm. of histamine per 100 grams body weight, and in some cases the initial blood pressure was higher and the responses to histamine not so marked as in the case of untreated suprarenalectomized rats. Three of this series had gained weight since operation and were found to have gross accessory cortical tissue. Disregarding these, the initial blood pressure range was from 40 to 128 mm. Hg, averaging 80 mm. Three cases had initial pressures above 100 mm. Hg. The similarity of the results in the daily treated and singly treated series indicates that the final injection of adrenalin may, in some cases, exert a pharmacological effect on the subsequent vascular reactions.

Discussion. In the results reported above there is some evidence to support and nothing to refute our original conclusion, namely, that the influence of the suprarenal medulla in protecting the rat from the fatal effects of histamine poisoning is effective by virtue of the physiological antagonism between secreted adrenin and histamine. This conclusion also gains support from the work of others, notably that of Dale and his collaborators (1929) who have produced much evidence for a special physiological antagonism between adrenin and a dilator principle, which probably is histamine. No evidence was found to support the suggestion that the protective action of adrenalin is dependent on a continuous effect maintaining the nutrition or tone of some part of the vascular neuromuscular mechanism. The failure of repeated injections of adrenalin to modify materially the vascular responses of suprarenalectomized rats to the larger doses of histamine does not favor this view. The appearance of increased susceptibility to histamine immediately after the removal of the influence of the medulla from the circulation also militates against the continuous support idea. This finding is opposed to that of Perla and Marmorston-Gottesman, as is also our repeated observation that increased susceptibility to histamine after suprarenalectomy is constant, at least up to five months after operation (Wyman, 1928). Their criticism that

we "failed to keep the factor of time interval between operation and injection of histamin constant" does not seem pertinent, inasmuch as the variations in time interval employed were designed to demonstrate the very point in question. Further evidence against the theory that adrenin is necessary to maintain the tone of the vascular mechanism under ordinary conditions is seen in our observation that the low blood pressure in suprarenal insufficiency is concurrent with cortical defect. This is in accord with many observations from other laboratories. Hoskins (1922) expressed the opinion that "it is doubtful whether the striking hypotensive effects of adrenal deficiency have their origin within the sympathetic system," and suggested that "the fault, if connected in any way with adrenin deficiency, lies in the effector rather than the conductor mechanism," and furthermore that "the more probable hypothesis to account for the low blood pressure which follows adrenal destruction is that not the medulla but the cortex is at fault." The lowest pressures are seen in those animals having severe symptoms considered to be indicative of cortical insufficiency, and only serve to emphasize still further the resemblance between the final stage of suprarenal insufficiency and the results of secondary shock. There is now much evidence that failure of the vascular mechanism, albeit secondary, is a prominent part of the picture of fatal cortical deficiency. Although the suprarenal medulla does not seem to afford a steady maintenance factor, as does the cortex, its importance in maintaining vascular tone in times of stress, such as during shock of various types, appears to be established. We still believe, furthermore, that it is able to do this immediately when called upon, without the necessity of previous continuous action.

In this as in previous studies the fact that completely suprarenalectomized rats and rats possessing apparently functional cortical tissue but no demonstrable medullary tissue respond in the same way to injected histamine is amply demonstrated. Although the subcutaneous injections of adrenalin did not materially affect the responses to histamine, it has been pointed out before (Wyman, 1928) that intraperitoneal injections of sufficient amounts will afford some measure of protection, and that at the best injections are a very imperfect imitation of the normal secretion. Subcutaneous injections failed to protect in the previous study. The fact that Perla and Marmorston-Gottesman found protection in 50 per cent of cases with injected adrenalin points to lack of the medulla as the important factor in increased suceptibility. Their criticism that of our previous series treated with single injections of adrenalin the percentage of survival was about the same as that of untreated suprarenalectomized rats, and that "experimental conditions—were not kept constant," was evidently based upon an interpretation of our table as a whole. The table gave all the experimental data without selection of favorable cases.

A perusal of the text in conjunction with an inspection of the table would show that variations in the amounts of adrenalin and histamine injected were deliberately used in a search for a protective dose, that partial or complete protection was found with sufficient doses, and that the failure of certain smaller doses to protect was recognized and adequately discussed.

SUMMARY

1. Increased susceptibility to histamine in suprarenalectomized rats follows immediately the removal of the influence of the suprarenal medulla from the circulation.

2. Direct microscopic observation of intestinal or ear blood vessels showed only dilator responses to histamine, applied directly or intravenously. Arteries, veins and capillaries dilated in the order given.

3. The blood pressures of suprarenalectomized rats having transplanted or gross accessory cortical tissue, but no demonstrable medullary tissue were similar to those of normal rats. The blood pressures of suprarenalectomized rats having no cortical tissue were distinctly lower, indicating that the low pressure seen in suprarenal insufficiency is concurrent with cortical rather than with medullary defect.

4. The minimal dose of histamine which would produce a detectable fall of blood pressure was about 0.0001 mgm. Little difference could be seen among the various types of rats with respect to the responses to small doses of histamine which produce small transient depressor responses (0.0001 mgm. to 0.001 mgm. per 100 grams body weight).

5. The minimal dose of histamine necessary to produce a permanent fall of blood pressure is from 0.3 to 0.5 mgm. per 100 grams body weight for normal and blank operated rats, and from 0.05 to 0.1 mgm. per 100 grams for suprarenalectomized rats with or without transplanted or gross accessory cortical tissue.

6. Subcutaneous injections of adrenalin, twice daily during seven days following suprarenalectomy, did not materially affect the vascular responses to histamine. Such slight differences as did appear were also seen in a series which received a single subcutaneous injection of adrenalin prior to the injection of histamine.

7. It is believed that the influence of the suprarenal medulla in protecting the rat from the fatal effects of histamine poisoning is effective by virtue of the physiological antagonism between secreted adrenin and histamine, rather than that the protective action of adrenin is dependent on a continuous effect maintaining the nutrition or tone of some part of the vascular neuro-muscular mechanism.

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STUDIES ON THE ELECTRICAL STUNNING OF DOGS[1]

A. C. IVY AND F. S. BARRY

From the Department of Physiology and Pharmacology, Northwestern University Medical School

Received for publication July 20, 1931

This work was undertaken after one of us (Ivy) was invited to view the experiments on the electrical stunning of hogs being conducted at the packing plant of Swift & Company. When the current was applied to the hogs, they apparently lost consciousness, and it was possible to suspend and bleed them without the usual struggle or outcry. The question arose as to whether the hogs so "stunned" were actually rendered unconscious and analgesic or could not squeal because of a spasm of the vocal cords or inhibition of respiration. It was decided to search for the answer to this question by undertaking experiments on dogs because their reactions are so well known and because they can be subjected more readily to experimentation on account of their size.

The literature on electric analgesia and sleep is reviewed thoroughly by Robinovitch (1914). Although the phenomenon was discovered by d'Arsonval in 1890 it was not seriously studied until Leduc and his colleagues began their work in 1902. Their findings were essentially as follows: Using a wheel interrupter and large capacity storage batteries, they found that a current of from 40 to 80 volts and from 0.5 to 4 milliamperes interrupted at a frequency of 6000 per minute and in such a manner that the current was on one-tenth of the total time when applied to a dog with the electrodes on the back of the head and base of the spine produces a sleep-like state which continues throughout the duration of the current and for a few minutes thereafter. They report that some rigidity occurs on application of the current, but disappears as the current is continued. Leduc permitted his two assistants, Rouxeau and Malherbe, to attempt to induce anesthesia in him, but the experiment was discontinued before anesthesia was complete. He reported, however, a loss of motor function, a dimming of vision, decreased hearing, and a sensation of numbness.

In our work we were primarily interested in producing a rapid anesthetic effect or stunning of animals from which they would recover, and in study-

[1] This work was made possible by a grant from the American Institute of Meat Packers.

ing 1, the completeness of the anesthesia; 2, the duration of the anesthesia following withdrawal of the current; 3, the effects of the current on various physiological mechanisms; and 4, in learning more of the mechanism of the anesthesia.

METHODS. A revolving wheel composed of five insulated segments and driven by a D.C. motor was used to interrupt the current. The brushes were adjustable so that the time of passage of the current could be varied. The current supply to the animal consisted of large storage batteries.

The following types of current were used to produce a stunning effect: 110 v, 6000 f, 3/10 on; 96 v, 6000 f, 3/10 on; 84 v, 6000 f, 3/10 on; 84 v, 6000 f, 1/10 on; 72 v, 6000 f, 1/10 on; 72 v, 6000 f, 3/10 on; 72 v, 9000 f, 3/10 on; 60 v, 6000 f, 3/10 on; 60 v, 6000 f, 1/10 on; 60 v, 9800 f, 1/10 on; 25 v, 6000 f, 3/10 on; 12 v, 6000 f, 1/10 on.

The following currents were used to produce a spinal analgesia: 10 v, 6000 f, 1/10 on; 8 v, 6000 f, 1/10 on; 6 v, 6000 f, 3/10 on; 6 v, 6000 f, 1/10 on; 4 v, 6000 f, 1/10 on.

Several groups of experiments were performed. In the first group, dogs were anesthetized with barbital and subjected to the current, the amount, frequency and position of the electrodes being varied. Tracings were taken of respiration and blood pressure. This was repeated on animals whose vagi had been cut. In the second group, unanesthetized dogs were subjected to the same procedures as in group one and tested as to "consciousness," loss of pain sensations, duration of the results of stimulation and reflexes dependent upon cerebral activity. In the third group, unanesthetized dogs were subjected to the same current used above, except that the current was applied by placing the animals in a tank of water through which the current passed. This method was also used on cats, rats and fish. In the fourth group, all the above experiments were repeated using current from a series of laboratory inductoria.

Contact to the animal was made by placing small brass binding-posts wrapped in gauze saturated with salt solution in the ears of the animal or by tin plates wrapped in gauze applied to shaven areas on various parts of the body.

Figure 1 shows the oscillogram of the current delivered by our interrupter.

RESULTS. Group I. *Effect of various currents on blood pressure and respiration in anesthetized dogs:* All the types of current listed above were used. In studying the effects of the current on cardio-circulatory and respiratory mechanisms, it was found that if electrodes were placed in the ears of the animals, the reaction described below invariably took place with any current over twelve volts, and was seen with any current producing marked rigidity of the animal when applied to the back of the head

and the lumbar spines. The same effects were obtained if one electrode was placed low on the back and the other at most any point on the head provided a current of sufficient voltage was used.

On closure of the circuit there is a sudden fall in blood pressure, the amount of fall being dependent on the strength of the current. Respiration is either arrested in deep inspiration or becomes very forced and rapid. If the application of the current is continued, the respiration remains arrested or rapid depending on the strength of current; but the blood pressure soon returns to or above the normal level, the pulse showing rather typical "vagus beats" which continue for varying periods of time.

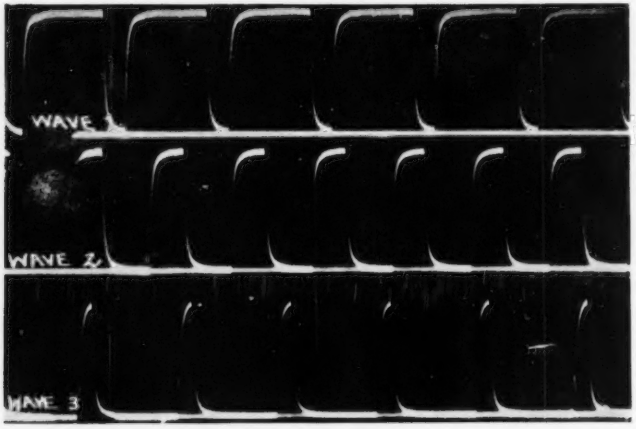

Fig. 1. This figure is an oscillogram made by the current delivered by our interrupter. The motor speed was 1000 per minute which yields 5000 impulses per minute, since our contact wheel was made up of five segments. The non-inductive load resistance was 660 ohms. The change in the tracing was made by varying the position of the brushes so that in wave 1 the current was on 0.8 of the time; in wave 2, 0.6; and in wave 3, 0.2. With 60 volts wave 3 causes "stunning;" wave 1 does not.

A second stimulus thrown in before the animal has returned to normal has less effect unless the time of stimulation or amount of current is increased. Quite frequently the fall is followed by a temporary rise in blood pressure.

After cutting both vagi, on application of the current, the blood pressure was found to rise instead of to fall. This was interpreted to mean that in the intact animal the stimulation of the cardiac inhibitory center was predominant, and that when its effect on the heart was removed by sectioning the vagi the cardio-accelerator and vasomotor centers were stimulated resulting in the rise in blood pressure.

Fourteen dogs were used in this group of experiments. With the vagi intact the average fall in blood pressure was 64 mm. Hg. With the vagi

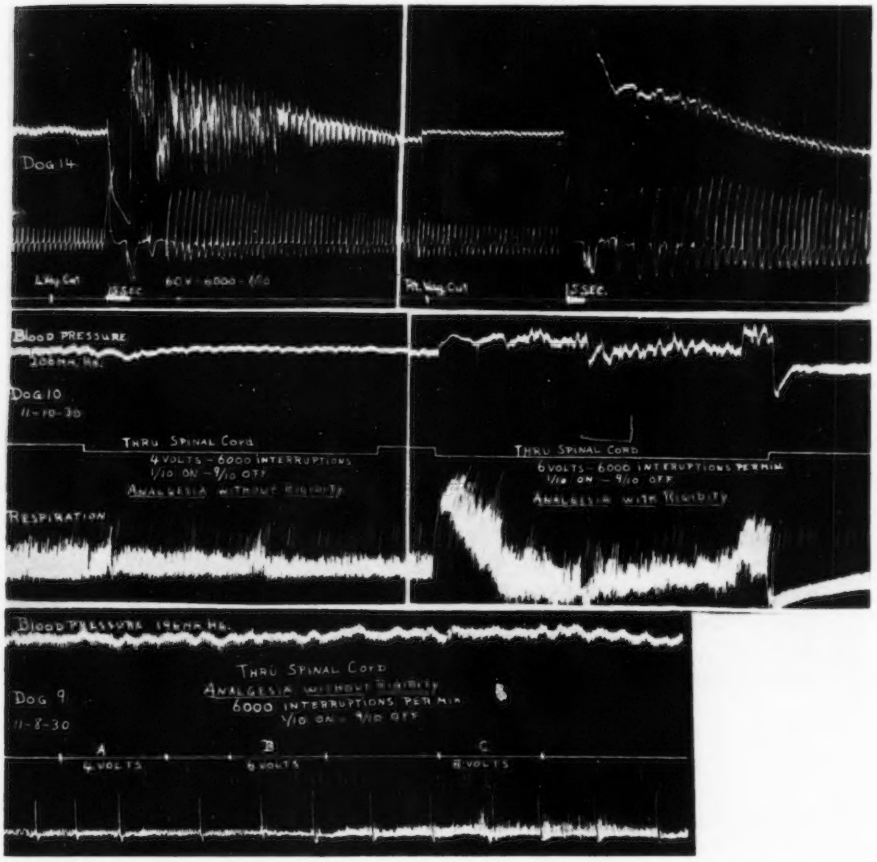

Fig. 2. The upper tracing shows the effect of the current (60 v., interrupted 6000 times per minute, current on one-tenth and off nine-tenths) on blood pressure and respiration after section of left vagus and then after section of the right vagus. The typical inspiratory arrest did not occur in this dog.

The middle tracing (dog 10) shows the effect of passing the current with low voltage through the spinal cord for spinal analgesia. It is to be noted that in this tracing 4 volts produced analgesia without influencing blood pressure, respiration and muscular tone, but when the current was increased to 6 volts rigidity resulted which was accompanied by changes in blood pressure and respiration.

The lower tracing (dog 9) shows that the voltage may be changed in some dogs without producing rigidity and material changes in blood pressure and respiration.

sectioned, the average rise was 86 mm. Hg (fig. 2, upper tracing). There was apparently no difference in the reaction in the normal dog to high or low frequencies (from 2200 to 9800 per minute) or to current which passed three-tenths rather than one-tenth of the time.

Direct current (70–110 v) uninterrupted caused a marked rise in blood pressure.

When the electrodes were placed on the back of the neck and lumbar spines and low voltages (4–12 v) used to produce an analgesic rather than a stunning effect, no change in blood pressure was noticed until sufficient current was used to produce rigidity of the muscles. Respiration, however, was augmented in certain instances (fig. 2, lower tracings).

In order to ascertain whether the current was stimulating the vagus nerves or the cardio-inhibitory center in the medulla, the electrodes were placed on the peripheral ends of the cut vagi. The current was applied for 15 seconds. The resulting fall in blood pressure averaged 60 mm. Hg. When this was repeated on unanesthetized dogs, there was no stunning or anesthetizing effect. In these experiments the vagi were exposed under ether anesthesia. No unpleasant responses were elicited by this procedure.

The brain was exposed on three anesthetized dogs. Direct observation of the cerebral vessels during application of the current failed to reveal any constriction or dilatation. This further substantiates the claim that the effects of the current were not due to cerebral anemia. (In one dog the current was applied with the usual results of fall in blood pressure, marked rigidity, etc. Decerebrate rigidity was then produced by decerebration. Application of the current now relieved the rigidity which reappeared when stimulation was discontinued. This was repeated several times.)

The experiments in this group were performed because of the possibility that the anesthetizing action of the current might be due to the cerebral anemia resulting from the marked fall in blood pressure. It can be concluded that the loss of consciousness that occurs in unanesthetized dogs on the application of this current is not due to a fall in blood pressure due to excitation of the cardio-inhibitory center.

Group II. *Certain effects of the various currents on unanesthetized dogs:* The animals in this group were subjected to various types of current as in group I. The following observations were made: *a*, time of return of an apparently conscious reaction to painful stimuli, or length of period of analgesia; *b*, time of return of "climbing reflex;" *c*, time of return of "drop reflex;" *d*, time of return of conscious proprioception; *e*, time of return of normal gait.

These were selected as being the most reliable criteria of cerebral activity. The corneal and patellar reflexes were not used because of the frequency of convulsions immediately following the cessation of the shock,

which made accurate observations impossible. It may be said, however, that the pupillary and patellar reflexes were lost but returned a short time before a reaction to pain could be elicited.

To elicit pain the animals were pinched on the pads of the feet, the tail or the ears.

"Loss of consciousness" was taken as the state in which the animal would not react to painful stimuli in an apparently conscious manner. The other tests or reflexes elicited as mentioned above were regarded only as tests of cerebral activity.

It is impractical to publish the tabulation of our data showing the variations from animal to animal, and the variation in the same animal, which occurred on using the various currents listed above. Only the essential features of our observations will be recorded.

Regardless of the type of current used for "stunning" purposes, a loss of reaction to painful stimuli resulted lasting from 55 seconds to 12 minutes in every dog (40 dogs). In practically every instance when the dog finally responded to a pain stimulus, he became immediately sensitive to further such stimuli, i.e., the passage of the dog into the pain responsive state was immediate, and not gradual. In other words, the animal was aroused by the pain stimulus as a sleeping dog is aroused. The other reflexes would either return immediately or in quite rapid sequence. If a pain stimulus was not applied, the recovery of the other reflex activities was definitely delayed. This means that the anesthesia induced by the electric current is quite different from ether anesthesia. However, after being aroused by a pain stimulus, the animal would often remain in an apparently dazed state for a much longer time—but urging or slightest pain would show that the animal was essentially normal.

The typical reaction to the stimulation was as follows: On closure of the circuit the animal becomes rigid, the legs and neck being in extensor spasm, and immediately falls on its side. If the current is left on for a period exceeding 30 seconds, there may be tremors of the jaws and legs. Respiration is stopped in deep inspiration and incontinence of feces and urine results. On opening the circuit there is immediate relaxation and several slow but deep respiratory movement usually occur. Then convulsive movements may start which may amount only to one or two kicking movements or may last for several minutes as a "running fit." Whether these fits are present or not seems to have no influence on the average time of return of the reaction to a pain stimulus. All dogs recovered.

The average length of time for appearance of reaction to pain (40 dogs) after withdrawal of the current was 2 minutes and 8 seconds—the shortest time being 55 seconds and the longest being 12 minutes. The longest average for any one type of current was 4 minutes and 14 seconds obtained with 72 volts passing $\frac{3}{4}$% of the time for 15 seconds. The same

current passing $\frac{8}{10}$ of the time causes rigidity but no after-period of analgesia. Other conditions the same, the $\frac{3}{10}$ type of current gave the longest after-period of analgesia in the dog.

The current was applied for different lengths of time in order to ascertain the minimum and optimum time for anesthesia. It was found that in order to secure an anesthesia of 1 minute duration uniformly, the current must be applied for a minimum time of 10 seconds. The optimum time was found to be 15 seconds. Applying the current for a longer period of time did not prolong the anesthesia.

In using the current for spinal analgesia rather than stunning, the tin electrodes were placed on the back of the neck and lumbar spines and much smaller voltages (4-12 v) used. The animals were tested for susceptibility to painful stimuli, rigidity of limbs and abdomen. During this type of stimulation the animals do not lose consciousness. Respiration may be augmented or not changed, and the pulse rate is usually slower than normal. The optimum current varied for each animal and can only be obtained by gradually increasing its strength until the desired effect is produced.

In general it may be said that with this type of current, if it is sufficient to cause analgesia, it produces too much rigidity for practical use. This was attributed to the imperfection of the interrupter which caused it to produce a faradization effect.

In two dogs stunning was repeated daily for 6 days and the animals observed for permanent effects of this procedure. As far as could be observed, the animals were entirely normal at the end of one month. One dog was subjected to the spinal analgesic current 10 minutes daily for 6 days and likewise remained normal.

Group III. In this group of experiments the animals were placed in a wooden tank filled with water through which the current was passing in an attempt to find a more convenient manner of making contact with the animal. This was done for practical or commercial reasons.

It was first tried on fish (2 v) using ordinary medium sized goldfish, and found to be very effective. Rats (25 v) and cats (25 v) were also used with good results. Electrical stunning of fish is easily demonstrated by passing the current from a laboratory inductorium through the bowl containing them.

In using dogs no appreciable difference could be shown between the results of this method and direct application of the current through the ears provided the animal was completely submerged. If removed immediately on opening the circuit, the animal does not inspire water as respiration is not resumed for a few seconds.

Group IV. When the above attempts to produce stunning of the animals were repeated using current from a series of four laboratory inductoria, the results were practically the same with the exception that the

pain stimuli caused a reaction slightly sooner than with the other type of interrupted current.

Discussion. Our results show quite decisively that the stunning currents used render the dogs analgesic for a short period (55 seconds to 12 minutes) following the withdrawal of the current. Although it is obviously impossible to state that the animals are unconscious during this period, there being no absolute test of consciousness in lower animals, we are convinced, as were Dr. A. J. Carlson and others who viewed some of our experiments, that the animals are unconscious during the period of analgesia. The inability to elicit the "drop reflex" and the "climbing reflex" during this period and even for a longer period, if a pain stimulus is not applied, definitely demonstrates that the motor cortex is not functioning in a normal manner. It is well known that in man the passage of strong alternating currents through the body in amounts insufficient to cause death cause unconsciousness or "electric shock." The fact that the dogs began breathing soon after the withdrawal of the current demonstrates that their failure to cry out on the application of a pain stimulus is not due to an inhibition of respiration of a spasm or paralysis of the vocal cords. This is further shown by the fact that the application of a pain stimulus did not even elicit a spinal flexion reflex during the greater part of the anesthetic period. We are convinced that the evidence of loss of consciousness is strongly presumptive, if not absolute.

How the electrical current produces anesthesia cannot be stated. It is quite apparent from our results, however, that a cerebral anemia induced by a fall in blood pressure (64 mm. Hg) due to the stimulation of the cardio-inhibitory center is not the cause, since stimulation of the peripheral vagi by the current for 15 seconds, which causes an analogous fall in blood pressure, does not induce anesthesia nor an apparent loss of consciousness, and since a second application of the current soon after the animal has recovered (4–5 min.) causes only a slight fall in blood pressure (30 mm. Hg) but an identical period of anesthesia and an apparent loss of consciousness as occurred after the first application of the current. The anesthesia is obviously not due to an injury or "burning out" of the nervous tissue because the current can be applied several times a day for a period of a week without inducing permanent effects. The interruption of the direct current is essential for the production of the anesthesia, since uninterrupted direct current does not induce anesthesia; neither does it induce as much rigidity. The facts that the stunning current causes marked rigidity, a spastic or tonic extensor spasm, the attitude of "decerebrate rigidity," that a faradaic current of adequate strength from laboratory inductoria causes a marked rigidity with analgesia, and that human beings suffering from a very severe attack of Jacksonian epilepsy lose consciousness and are anesthetic, suggest that the mechanism involved in

the loss of consciousness in "electrical stunning" is the same as that involved on strong electrical excitation of the cerebral cortex. However, the observations, which confirm Leduc and Rovinovitch, that the frequency of interruption and duration of the current play a rôle in the induction of anesthesia and that a "stunning current" of low voltage (12–20 v) when applied throughout the length of the spinal cord induces analgesia in some dogs without rigidity, indicates that a block of the reflex pathway may be produced by the current.

We believe that the induction of spinal anesthesia with appropriately interrupted direct current has considerable promise and warrants further investigation. Such an investigation awaits the perfection of an apparatus that is free of faradization effects or one that yields an oscillographic curve with a square plateau and adequate voltage. It is believed that such an apparatus would not produce the rigidity seen in the majority of dogs on the application of the current to the spinal cord and would render surgical operations feasible.

CONCLUSIONS

1. The various types of current used will stun dogs when appropriately applied.

2. The average duration of the "anesthesia" after withdrawal of the current is 2 minutes and 8 seconds. Loss of consciousness is strongly presumptive.

3. The most efficient current is one of 72 volts—interrupted 6000 times a minute and passing $\frac{3}{10}$ of the total time, the application lasting fifteen seconds. The average time that elapsed before a pain reaction was elicited after application of this current was 4 minutes and 14 seconds. The shortest time was 2 minutes and the longest 12 minutes. The minimum effective time for application of the current is 10 seconds, the optimum being 15 seconds.

4. Interrupted current of the type used and passed through the brain causes a marked fall in blood pressure due to stimulation of the cardio-inhibitory center, and inhibition of respiration usually in the stage of marked inspiration. The fall in blood presure is followed by a rise due to stimulation of the vaso-constrictor center.

5. This fall in blood pressure does not produce sufficient cerebral anemia to account for the "loss of consciousness."

6. The application of the current inhibits cerebral activity as judged by the loss of reaction to painful stimuli and absence of the "drop reflex," "climbing reflex" and conscious proprioception for varying periods of time, depending on the type and duration of the stimulus. This inhibition continues for several minutes after breaking the current.

7. Tetanizing current from laboratory inductoria will cause similar stunning effects, if applied to the brain.

8. Adequate contact with the animal can be made by means of tap water through which the current is passing.

9. Spinal analgesia can be produced in dogs by placing one electrode on the back of the neck and the other over the lower lumbar region and passing a current of 4 to 12 volts, interrupted 6000 times per minute, one-tenth on and nine-tenths off. This analgesia is accompanied with more or less muscular rigidity in most dogs. When applied daily for one week it caused no disturbances of the function of the spinal cord.

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GRAPHIC ANALYSIS OF FROG HEART TRACINGS

COLIN C. STEWART

From the Department of Physiology, Dartmouth College

Received for publication August 14, 1931

The tracing obtained from the beating frog's heart recorded by the suspension method is a curve compounded of the records of the rhythmical contractions of sinus, auricles, ventricle and bulbus, each responsible for its regular proportion of the total shortening indicated by the movement of the lever.

There are both mechanical and mathematical methods by which an analysis of a compound tracing might be made. Since the four components of the frog heart tracing all have the same period the mathematical method (Fourier series) is not applicable, and as to the mechanical methods Miller (1916) has found in his treatment of the analysis of sound waves that where the number of components is limited it may not be necessary to provide a machine for performing the analysis (p. 133). We have therefore attempted to make use of the graphic method.

As a preliminary to all analyses the tracings have been re-drawn with vertical coördinates to eliminate the distortion introduced by the recording lever, and the drawing has been made on an enlarged scale, usually $\times 5$ or more, to facilitate the work of drawing and to reduce errors of measurement. The correction and enlargement of the tracing are readily accomplished by the use of a transparent rule on which are inscribed horizontal lines two millimeters apart, and a vertical arc with the same radius as the recording lever. It is a simple matter to find the level at which the arc fits the check lines recorded with the drum at rest. The rule may then be moved right and left to read both the height and the time relations of any point on the tracing, and the readings are plotted on rectangular coördinate paper.

In a study of the compensatory pause already published (1931) we have found that the responsive period in ventricle, auricles and sinus is quite constant, particularly as to the termination of the responsive period in each division of the heart. Since the end of the responsive period is the beginning of the corresponding contraction phase it thus becomes possible, by mapping the responsive periods, to determine with comparative accuracy the time of the initiation of the beat of sinus, auricles and ventricle. The beginning of the responsive period is equally clearly the beginning of the

relaxation in each division of the heart. Starting with a cardiogram for which we know the responsive periods for sinus, auricles and ventricle we can place the contraction of each of the components of the compound contraction in its proper position in time. To determine the relative extent (height) of the separate contractions one may depend on trial alone, or one may get further data by observing tracings in which, as a result of artificial stimulation, the separate waves may appear separately or in new combinations. The deduction from the total height for the small contraction of the bulbus arteriosus we have made usually by trial alone, though occasionally the bulbus tracing emerges distinctly at the top of a wave made wider than usual because of increased fulness of the heart, as in figure 2c, or in figure 5.

Figure 1 shows the method in detail. A cardiogram is shown re-drawn with vertical coördinates. The beats of the series are numbered from 1 to 7 for reference. In the first three the responsive periods are plotted in heavy horizontal lines.

The first step in the actual analysis of the curve is the subtraction from the vertical measurement of each succeeding compound wave of the contraction assigned to the bulbus arteriosus. The areas so subtracted are lettered *B.B.*

For the sinus, perpendiculars are dropped from the end of the lines indicating the sinus responsive periods in beats 1 or 2. This determines the beginning of the sinus beat and the sequence. A similar perpendicular line from the beginning of the sinus responsive period in beat 2 marks the beginning of the sinus relaxation. To determine the form and duration of the relaxation phase a trial line, *X*, is drawn to eliminate the variation in the original tracing due to relaxation of the sinus, and the vertical components of the space so enclosed give the data for plotting the sinus tracing from the beginning of the relaxation to its end. The remainder of the sinus wave, the ascending limb of the curve, is determined by trial. When once determined the sinus curve is repeated at the proper intervals, and is then subtracted from what remains of each beat after subtraction of the bulbus. The areas deducted for the sinus contraction are lettered *S.S.*

The auricular component is then derived in the same way. Perpendicular lines drawn through the end of the auricular responsive period in beats 2 and 3 indicate the beginning of the auricular contraction and the auricular sequence. The beginning of the auricular relaxation is found by dropping a perpendicular from the beginning of the auricular responsive period in beat 3. The form and duration of the auricular relaxation are obtained by drawing a trial line, *Y*, to eliminate the auricular relaxation from the original tracing. The remainder of the auricular curve is filled in (broken line) by trial. When determined the auricular wave is repeated at the proper interval and is subtracted from what remains of each beat after

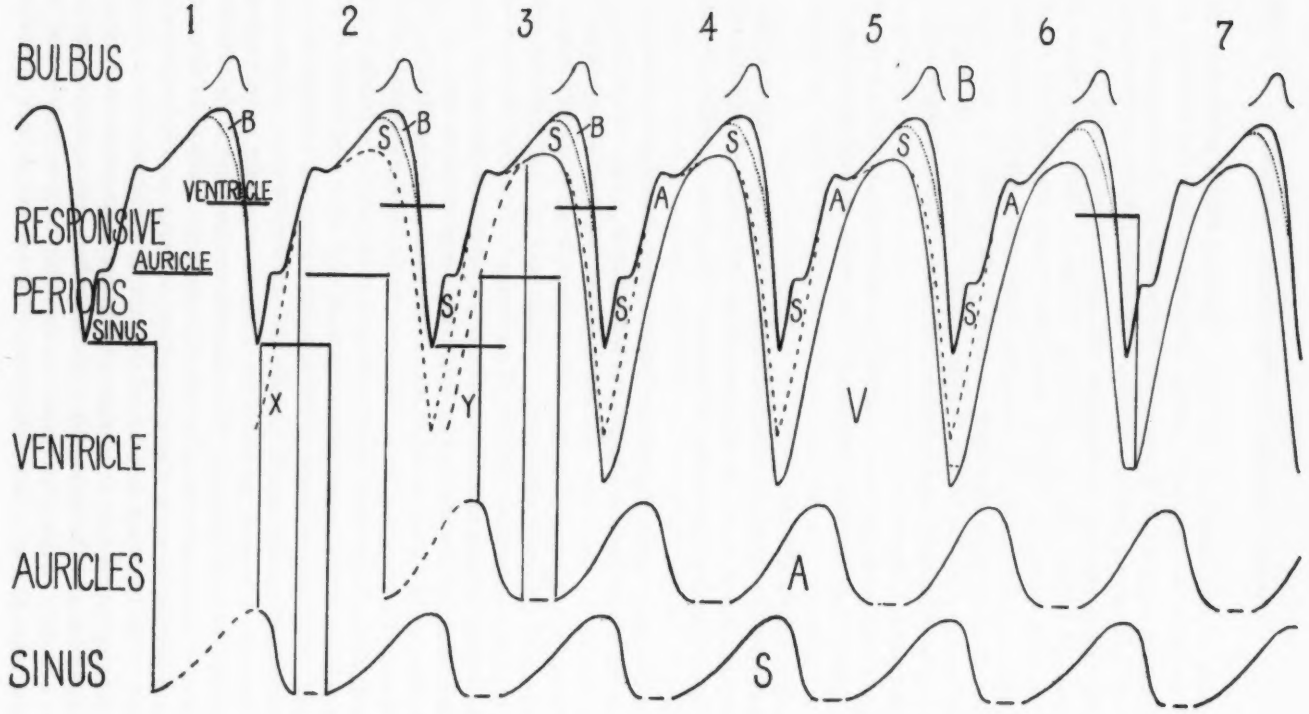

Fig. 1. Stages in the graphic analysis of a frog heart tracing. See text.

subtraction of the bulbus and the sinus. The area so subtracted is lettered *A.A.* The final remainder is the contraction of the ventricle.

No allowance has been made for the inertia of the lever and the elasticity of the heart muscle. On the ascending limb of the normal tracing, checked as it is at two points, first by the relaxation of the sinus and again by the relaxation of the auricles, it is probable that the record expresses very nearly the actual degree of shortening in the various divisions of the heart. There is no doubt, however, that the downward sweep of the lever during the relaxation of bulbus and ventricle carries it lower than its true position as an indicator. By the method of analysis herein described the error involved is allowed to accumulate and appears only in the resolved tracing of the ventricular contraction. It thus follows that the notch between ventricular beats is no doubt deeper than it should be. A correction is suggested in beats 6 and 7 of the diagram (fig. 1). This has the effect of allowing for a short period of "diastasis," and of making the beginning of the ventricular contraction curve coincide in time with the end of the ventricular responsive period, as it should. It may also be suggested that the resting level of the lever during brief inhibition of the heart, and the resolved level of the ventricular component during a long compensatory pause, afford some additional clues as to where the resting level of the ventricular curve really lies.

Where the form of the resolved curve for any part of the heart is determined by trial an attempt is made to adjust the separate components so that all shall have the same general form. A heart that is quick or slow, bloodless or full, should show the same characteristics in each of the four waves into which the compound tracing is resolved.

Attention has been directed to the error due to the inertia of the lever and the elasticity of the suspended heart. There is a second error, still smaller in amount, perhaps, but of importance when we attempt to deduce from the resolved tracings the exact form of the curve of contraction of the sinus or the auricles considered as spontaneously beating heart muscle. There is no doubt that by graphic analysis one can arrive at a very close approximation of the form of the shortening due to contraction, for example of the sinus. The shortening caused by contraction of the sinus has however two components. The major part of the shortening is to be ascribed to the shortening of the vertical length of the sinus itself but a minor part is played by the change passively induced in the flaccid auricles by the mere receipt of blood from the contracting sinus. Lateral distention of the auricles must slightly decrease their vertical dimension. The same must be true of the auricular curve, and while no doubt the curves worked out for the auricles represent chiefly the actual shortening of the auricles in the vertical direction, some small fraction of the shortening credited to the auricles is in reality due to a coincident decrease in the vertical length of

the ventricle caused by the injection of blood from the auricles. We have not as yet attempted to determine to what extent this element must be reckoned with in assigning a correct form to either sinus or auricle curves. It does not seem to be involved in the contraction of the ventricle itself.

By such methods we have analyzed tracings varying widely with respect to the form of the curve, and in cases where variations have been introduced by artificial stimulation. Figure 2 shows the analysis of a record which appeared to have undergone marked variation in the course of an hour's time. Starting as an almost bloodless heart the beats became distinctly full with the recovery of vascular tone. The usefulness of the method is shown by the possibility of resolving these varying forms without changing

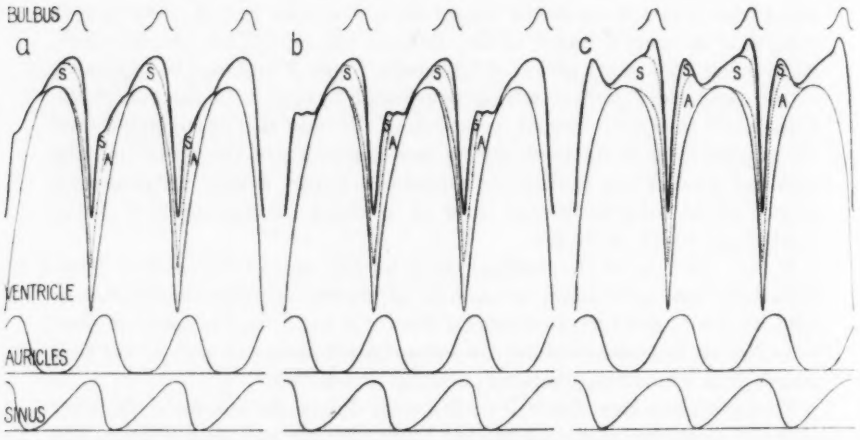

Fig. 2. Analysis of three forms of curve recorded by the same heart with varying amounts of blood.

the height of any of the four component waves and without altering the rate of conduction from sinus to auricles to ventricle, changing only the duration of the contraction (width of the wave) in each case, as would seem to be necessary to provide for the propulsion of increasing amounts of blood.

In any normal curve the analysis emphasizes certain facts that are quite frequently overlooked in the interpretation of the tracings. For example, the two minor waves on the ascending limb of the tracing do not mark the contractions of sinus and auricles respectively. They represent checks in the ascent of the lever caused by the relaxation of sinus and auricles. The contraction of the sinus begins just before, and the contraction of the auricles just after, the apex of the previous ventricular contrac-

tion. Occasionally the two relaxations are merged and only one notch appears, but the time of the beginning of the contractions of sinus and auricles does not vary to any marked degree. Again, after frequent stimulation of one auricle, there may be a slight dissociation of the beats of the two auricles, and the auricular relaxations are indicated by two such minor waves. The tracing shows therefore a total of three, with the sinus relaxation.

Worthy of note also is the fact that the duration of a single cardiac cycle greatly exceeds the time occupied by a single "beat" of the tracing. Thus the cycle that ends with beat number 4 in the chart (fig. 1) begins with the first shortening of the sinus early in beat number 3. In this particular case the ratio of cycle to beat is 1.58:1. Where the volume of blood is small or the rate of conduction slow the ratio may be 1.67:1 or even higher.

Again, it is evident that the apex of the ventricular contraction is earlier than the apex of the whole tracing. This accounts for the possibility of eliciting extra beats from the ventricle before relaxation seems to have begun.

The ventricle we have stimulated during the whole range of its responsive period to produce a series of "extra" or induced beats varying from the earliest to the latest possible. Only two of these are analyzed in figures 3 and 4 in which it will be observed that what appears to be summation in the case of early stimulation is due entirely to the piling up of the ventricular extra beat on the beats of the sinus and auricles, which have ordinarily passed their maximum before the ventricle is fully shortened. The later the stimulus falls, the less is the extra beat raised above the level of the regular beat preceding it.

In such series, running all through the responsive period of the ventricle we have never found any significant variation in the latent period. This is distinctly at variance with the oft-quoted finding of Marey (1876) who described longer latencies for the earlier stimuli. It should be evident that Marey's tracings do not show the ventricular response, but rather the response of the ventricle submerged beneath the sinus and auricular contractions.

The "extra" beat of the ventricle, according to the time of its occurrence, deals with a variable amount of the blood propelled by the auricles. Some blood is thus left in the auricles, and this in turn causes retention in the sinus, until the next normal sequence of the contractions of the various parts of the heart. An attempt has been made to resolve this congestion by dividing it among sinus, auricles and ventricle. It should be pointed out however that this is merely a matter of probability and does not affect in the least the analysis of the fundamental rhythms of the various parts of the heart.

For the auricles (fig. 6) there is shown an analysis of a typical extra

beat of the auricles followed by an extra beat of the ventricle. The wave of contraction cannot affect the sinus, which is itself in contraction and "refractory."

There is shown also (fig. 5) a tracing where early stimulation of the auricles results in an extra beat which, because the wave of contraction reaches the ventricle before it has begun to relax, is not followed by an extra beat of the ventricle.

For the sinus the analysis of the tracings showing the effect of stimulation is still simpler than for the other divisions of the heart. There is no obstruction of the blood stream by an extra beat of the sinus because the sinus beat is followed in regular order by the beats of the auricles and ventricle. The rhythms of the component parts of the heart are therefore clear cut and definite. It might be expected that the effect of artificial stimulation of the sinus would be to advance the next succeeding beat of the heart in its entirety, and so the whole sequence of beats. It is however quite evident in figures 7 and 8 that such is not the case. When a stimulus is applied to the sinus, even when applied so early as to be several times the length of the usual latent period in advance of the autochthonous beat, there is no change in the time of its origin. The sinus thus appears to be "refractory" in Verworn's sense (1913) even during diastole and until energy is accumulated for the next regular beat. The next beat of the sinus begins exactly when it would have begun had no artificial stimulus been applied. It is however a "quicker" beat, of shorter duration. Following the quickened beat, the next succeeding one is advanced. There is thus in these cases as a result of stimulation by a single induction current an effect produced on the sinus, and so on the whole heart after the lapse of more than a second. In general the advancement of the sequence is greater the earlier the stimulus is applied in the sinus responsive period.

We have shown the results of analyzing tracings of the normal frog's heart, and of the heart stimulated by a single induction current applied to ventricle, to auricles and to sinus. Other types of variation from the regular rhythm have been analyzed in the same way and the method seems to be useful in determining the course of events in any type of irregularity. It can be applied also in determining how much of the so-called "reaction" after vagus or crescent stimulation is due to the varying degree of superposition of the separate waves that is a feature of the return to normal after

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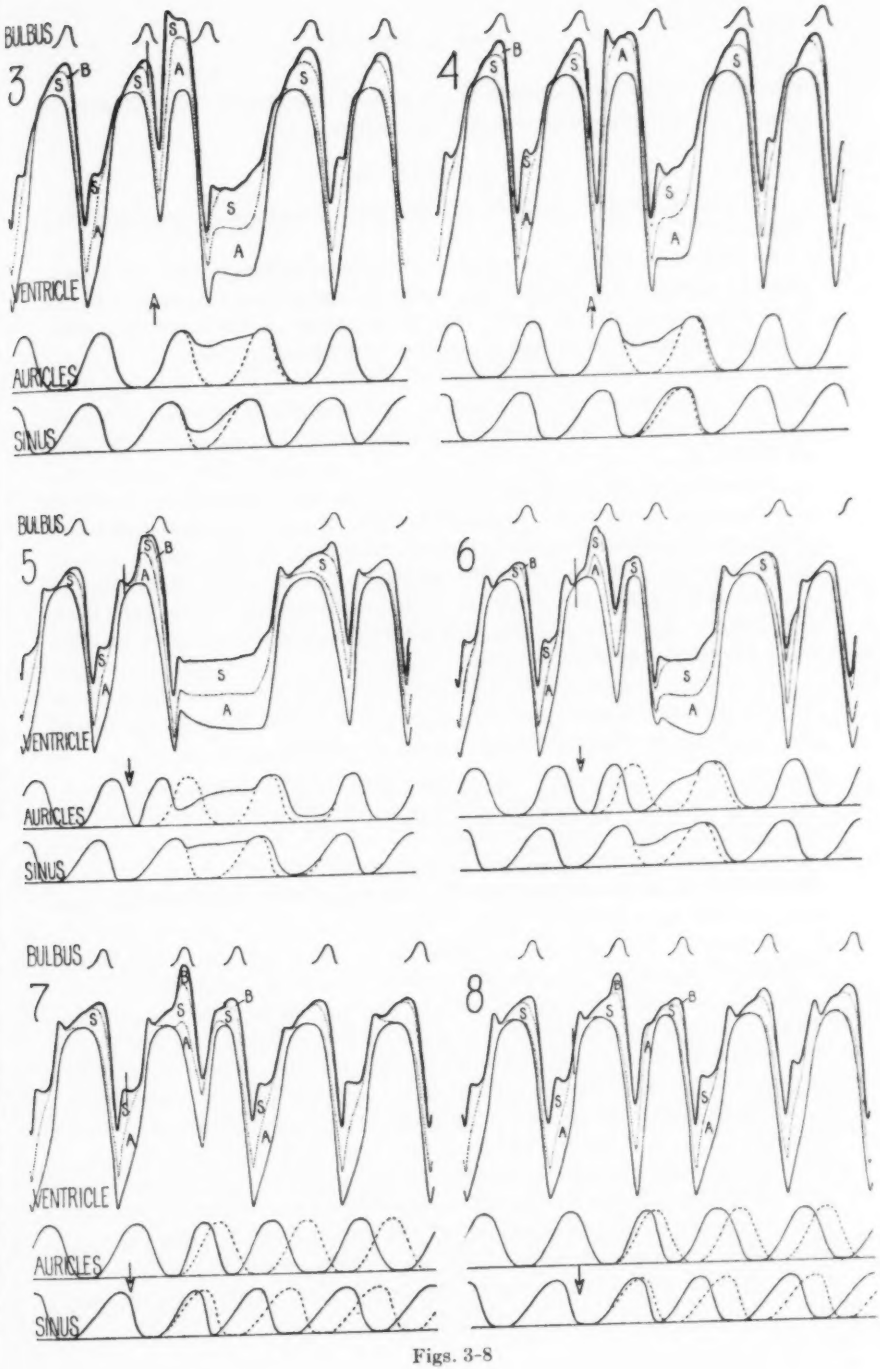
Figs. 3, 4, 5, 6, 7 and 8. 3. Early extra beat of the ventricle. The arrow marks the point of stimulation. 4. Late extra beat of the ventricle. 5. Early extra beat of the auricles, the wave blocked by the ventricular contraction period. 6. Typical extra beat of the auricles, followed by the ventricle. 7. The advance of the sequence caused by a single induction current applied to the sinus. The original rhythm is shown in broken line. 8. Advance of the sequence caused by later stimulation of the sinus, to show the relation between the time of stimulation and the degree of advance.



Figs. 3-8

inhibition because of variation in the rate of conduction, the dromotropic effect. Many other uses suggest themselves.

CONCLUSIONS

A method for the analysis of frog heart tracings is described and illustrated.

By this method it is shown that the sinus contraction begins just before, and the auricular contraction just after, the apex of the contraction of the ventricle in the previous cycle. The prominences on the curve frequently ascribed to their contractions are caused by their relaxations.

By analysis it may be shown that the ventricular extra beat never exceeds in total shortening the regular ventricular contraction. We have found no variation in the latent period whether the extra beat is early or late.

The auricular extra beat is followed by an extra beat of the ventricle except when the stimulus is applied early in the auricular responsive period in which case the ventricle frequently remains quiescent.

When single induction currents are applied to the sinus in advance of the autochthonous beat there is no change in the time of origin of the beat. The beat is quickened and the next succeeding beat is advanced in time, with the whole sequence of beats.

Other types of irregularity have been analyzed and the method seems to lend itself to clarifying many obscure points in the various responses of the frog heart to either direct or indirect stimulation.

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THE ABSORPTION OF TYRAMINE FROM THE INTESTINE

CHARLES L. COYLE AND T. E. BOYD

From the Department of Physiology and Pharmacology, Loyola University School of Medicine

Received for publication September 1, 1931

It has been shown (Hanke and Koessler, 1924) that bacteria normally present in the human intestine are able to form histamine and tyramine from the corresponding amino-acids. The absorption of the former amine has received considerable attention, that of the latter comparatively little. Histamine in amounts up to 200 mgm. can apparently be placed in any part of the normal gastro-intestinal tract of the dog without effect on the arterial blood pressure (Koessler and Hanke, 1924; Wangensteen and Loucks, 1928). Mammoser and Boyd (1929) confirmed this with respect to the normal intestine, but found that exposure of the mucosa to certain chemical agents may render it permeable to histamine. Ivy's experiments (1924) indicate that there is little absorption of unchanged histamine by the human intestine.

The results of experiments on cats have been different from those cited above. Mellanby (1915) using chloroform and A. C. E. anesthesia, obtained a marked fall of blood pressure on introducing histamine into the small intestine, though experiments on the colon were negative. Meakins and Harington (1922), with paraldehyde as the anesthetic, found that absorption took place from both the large and small intestine. Unpublished experiments by one of us (T. E. B.) on cats under ether anesthesia, gave results in entire agreement with those of Meakins and Harington. It would appear that, in respect to histamine absorption, there is a difference in behavior between the intestine of the dog and that of the cat. It should be added that MacKay (1930) using cats under chloralose and urethane anesthesia, found no evidence of absorption except when the histamine was administered in acid solution. It is possible, as she suggests, that the nature of the anesthetic may considerably modify the results obtained.

No such comparative data on tyramine absorption are available. Whether or not it can be absorbed from the intestine of any mammal, in sufficient amounts to produce a measurable effect, does not seem clear from the literature. The subject was briefly reviewed by Alvarez in 1924. While blood-pressure effects may be used as an index of absorption for both histamine and tyramine, the latter is the weaker in its

action, and larger amounts would have to be absorbed in order to produce a perceptible effect. The problem is also somewhat complicated by the probability (Ewins and Laidlaw, 1913) that tyramine carried in the portal circulation may be in part transformed in the liver into a pharmacologically inert substance.

PROCEDURE. The animals used were kept without food for 24 to 48 hours previous to experimentation. Ether was the anesthetic used with all of the 18 dogs of the series, and with 3 of the 9 cats. The remaining 6 cats were anesthetized with sodium barbital, 0.35 gram per kilo, given intraperitoneally. Both vagi were sectioned. Records were made of the respiration and of the carotid blood pressure. Tyramine hydrochloride

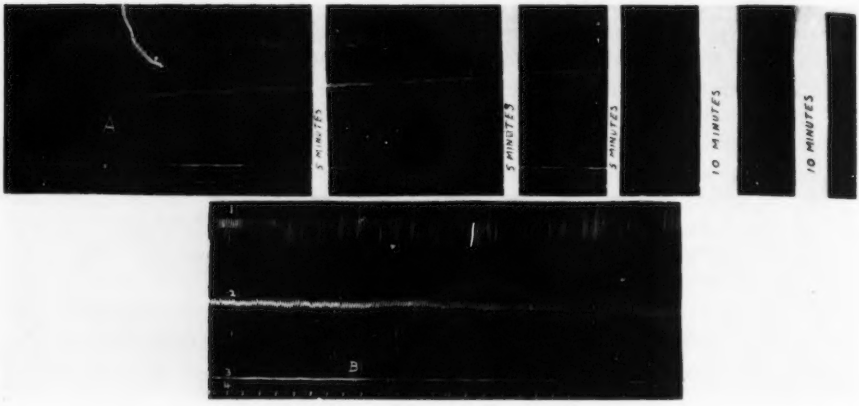

Fig. 1A. Dog. At *A*, injection into the cecum of 5 mgm. of tyramine hydrochloride per kilo. Time tracing, 30 seconds. The narrow white spaces represent intervals of 5 minutes; the wider spaces, 10 minutes.

B. Cat. At *B* injection into the cecum of 40 mgm. of tyramine hydrochloride per kilo. Time tracing, 30 seconds.

(Eastman) dissolved in 10 to 12 cc. of physiological saline solution, was introduced into the gut through a small rubber catheter. The catheter had been cut through about 4 cm. from the tip and the two parts joined by a short glass segment.

In the experiments on the large intestine, the catheter was inserted through an incision in the blind end of the cecum. The glass segment permitted a ligature to be drawn tightly around the gut over the catheter, holding the latter in a fixed position. For injections into the duodenum, the catheter was passed downward from an incision in the wall of the stomach and fixed in place by a ligature around the pylorus. The small intestine was not separated into loops for the study of absorption from its

several parts. Most of our injections were made into the cecum, since it is unlikely that significant amounts of tyramine would under natural conditions be found at a higher level.

1. *Absorption from the large intestine of the dog.* The arterial blood pressure of the dog responds readily to the administration of tyramine by this route. Doses of 5 mgm. or more per kilo always produced a definite rise. Of ten injections of 3 mgm. per kilo, only five gave clearly positive results. Figure 1A shows the effect of one injection. An equal amount of histamine similarly administered, is entirely without effect in the dog, although, given intravenously, histamine is by far the more potent of the two substances.

It is not possible from our data to estimate how much of a given dose of tyramine is actually absorbed under these conditions. To produce a measurable elevation of the arterial blood pressure, a larger amount is of course necessary than would be required if given intravenously. The duration of the effect, however, is much longer on intestinal than on intravenous administration. This is doubtless due in part to gradual absorption, but it is also partly due to the mechanical "cushioning" effect of the capillary circulation in the liver, as demonstrated by other workers in the case of histamine. Rapid injection of tyramine into a branch of the portal vein produces a blood pressure curve which is flatter and more prolonged than that seen on administration by way of a systemic vein.

2. *Absorption from the intestine of the cat.* In cats under barbital, the injection of 10, 15, 20, 25, 30, and 40 mgm. of tyramine per kilo, into the cecum, failed to produce any elevation of blood pressure (fig. 1B). Administration of 50 mgm. caused a slight rise, and injections of 30 and 40 mgm. per kilo, into the duodenum, gave positive results. With smaller amounts in the duodenum the results were negative or doubtful. In two of three cats under ether anesthesia, a slight rise of pressure was obtained, with 20 mgm. per kilo in the cecum.

For tyramine administered by way of the large intestine, therefore, the minimum effective dose in proportion to body weight is at least four times as large for the cat as for the dog. Such a difference does not appear with direct injections either into the systemic or the portal veins. The relative insusceptibility of the cat to tyramine administered by way of the intestine appears therefore to be due either to extremely slow absorption or to the action of some detoxicating mechanism in the intestinal mucosa. Koessler and Hanke (1924) present strong evidence for the existence of such a detoxicating mechanism for histamine in the dog, and their conclusion is strengthened by the recent studies of Best and McHenry (1930) on histaminase.

SUMMARY

The arterial blood pressure of the dog is readily elevated through the administration of tyramine by way of the cecum. The arterial pressure of

the cat, under similar conditions, is unaffected unless relatively large amounts of tyramine are used. This difference of behavior is the reverse of that demonstrated by other workers for histamine in the same species.

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RESPONSES IN THE ISOLATED LIMBS OF CRUSTACEA AND ASSOCIATED NERVOUS DISCHARGES

T. CUNLIFFE BARNES

Contributions from the Bermuda Biological Station for Research and Osborn Zoological Laboratory, Yale University

Received for publication August 19, 1931

The walking limbs and claws of decapod crustaceans have many properties which are particularly suited for experiments concerning excitatory processes, peripheral tonus and movements simulating reflex action. The most important features are the hinge joints, the simplicity of the muscular system consisting of a single flexor and extensor in each segment and especially the small number of motor nerve fibres. The opening and closing movements of the excised claw are well known from the early work of Richet (1882) and Biedermann (1895); a weak electrical stimulus produces opening of the claw and a stronger one is required for closing. Hoffmann (1914) has worked out the arrangement of the motor fibres in great detail and was able to prove by histological and physiological methods that the entire nerve between the second and third segments contains only five to six motor fibres. The more recent work of Segaar (1920) is not supported by other investigators. A. V. Hill (1929), when discussing the heat production and oxygen requirements of crustacean nerve points out that further research on these unmedullated fibres should throw light on the central nervous system of vertebrates which has several properties in common. The peripheral nature of the reflex phenomena in crustacean limbs including inhibition, rebound and reversal (as in the "scratch reflex") has been emphasized recently by Knowlton and Campbell (1929). There are no multiple synapses and a simple explanation postulating the accumulation of an excitable substance is suggested. Our present knowledge is largely confined to motor fibres but the writer (Barnes, 1930a) has called attention to the several interesting properties of the sensory fibres. In particular the dactylopodite (terminal segment) is an unique preparation, for there are no motor fibres present.

The present paper deals briefly with certain "reflex" movements exhibited by several species of crustacea observed in Bermuda during June and July 1931. The species which were identified from Verrill's paper (1908) include *Charybdella tumidula, Callinectes sapidus, Goniopsis cruentatus* and *Eupanopeus Herbstii*. A few observations were made on *Grapsus*

grapsus, Calappa flammea, and *Microphrys bicornutus.* The nerves of *Goniopsis* and *Grapsus* lose their excitability more readily than the other species; those of *Calappa* and *Microphrys* are rather difficult to dissect out. Verrill's paper may be consulted for notes on the natural history of these forms and photographs of the entire specimens and of isolated claws. The original object of the investigation was to study the sensory discharges produced by tactile and proprioceptive stimulation and to correlate tonic muscular contractions with discharges of motor impulses. In the course of this work curious movements of the isolated preparations were observed.

METHODS. The claw or walking limb was severed close to the body or the animal was induced to "shed" the appendage. The final preparation included no segments proximal to the meropodite or "femur." The entire nerve in the meropodite was dissected out with great care by means of fine glass hooks when possible. The dissections were made under sea water and the preparation allowed to stand in fresh sea water for about two hours. This immersion in sea water was found by Levin (1927) to prevent loss of excitability. Biedermann (1895) observed the perishable nature of crustacean nerves and not more than half of our preparations retained their excitability. The greater longevity of motor fibres mentioned in a previous paper (Barnes, 1930c) was confirmed in all preparations studied. In temperate climates the nerves of crabs are far more perishable in summer (Pantin, 1929) but it is not known if this seasonal variation occurs in Bermuda.

The nerve was placed on cotton points soaked in sea water leading from nonpolarizable sea water—AgCl—Ag electrodes connected with a resistance coupled amplifier of standard type. The writer is greatly indebted to Prof. A. Petrunkevitch who designed the instrument. Three UX-240 radio tubes were used, having a high amplification factor (Mu = 30) followed by a power tube, X-171 A (Mu = 3) leading to a loud speaker. This arrangement gave a faithful sound reproduction of both sensory and motor impulse discharges. In the absence of an accumulator, four dry cells supplied the filament heating. For subsequent analysis of results it is important to note that no electrical stimulation was employed. The afferent discharges were elicited by natural stimulation of sense organs, i.e., touching the hairs or bending the limb. The motor fibres were stimulated in some cases by cutting with scissors, by pressure or by stretching the nerve.

Proprioceptive impulses. In an earlier paper (Barnes, 1930a) it was reported that discharges of sensory impulses are set up by bending the limb in *Cancer pagurus* and in the lobster. These proprioceptive impulses were found in the walking limbs and claws of all the species of Bermuda crustacea studied. The presence of these afferent impulses affords important evidence supporting Crozier's (1929) "muscle tension" theory of geotropic orientation in arthropods. Crozier assumes that the direction of the crawling animal on an inclined surface is controlled by the body weight pulling

on the supporting musculature. The organism orients until the pull of the body weight is the same on both sides or more precisely until the difference of the bilateral tensions is reduced below a threshold value. It is clear that the scheme requires sensitive tension receptors responding to movements of the walking appendages. The theory was first established with rats in which proprioceptive mechanisms were previously known. The Θ angle of orientation (between the path of the animal and a line at right angles to the axis of the inclined plane) was found to be a rectilinear function of log sine α (α being the angle tilt of the substratum).

Similar relationships obtain in the case of several arthropods, including the fiddler crab *Uca* and several species of ants; with these organisms the formula becomes $\Delta\Theta/\Delta \text{ sine } \alpha = \text{const.}$ This interpretation of geotropism in anthropods is entirely confirmed by the discovery of proprioceptive impulse discharges in all the Bermuda limb preparations.[1]

The nature of the end organs responding to limb movements is not known but it is obvious that the tactile receptors in the skin[2] of the joints will be stimulated at each flexion and extension. On the ventral surface of the joints there is a large area of exposed skin which is folded or stretched when the limb moves. No sensory structures resembling the muscle spindles of vertebrates have been found in arthropod muscles[3] and Hoffmann (1914) states that the sensory fibres passing the musles of the crab's leg send no branches into the muscles. It is not improbable that the proprioceptive impulses arise in the joints only.

The rapid "adaptation" was a striking feature of all the Bermuda prepations. Following the well known terminology of Adrian (1928) rapid adaptation of a sense organ signifies that it does not produce a lasting discharge during the application of a persistent stimulus. A two gram weight was attached by means of a silk thread to the dactylopodite of the leg. During the fall of the weight when the joints were in actual motion afferent impulses were reproduced by the amplifier leading from the nerve in the meropodite. When the movement ceased, however, the discharge died away at once in spite of the tension maintained by the hanging weight. Slight oscillations of the suspended weight were sufficient to produce joint movements giving rise to fresh proprioceptive impulses. Clearly, then, the proprioceptors in crustacea might be termed "movement receptors" rather than "tension receptors." Recovery from adaptation is very rapid; a

[1] A few experiments were made in Bermuda on large cockroaches *Periplaneta*. Bending the leg produced sensory discharges thus corroborating the geotropism studies on insects.

[2] The book of Fortuyn (1920) may be consulted for descriptions of sense cells in crustacea. Vom Rath (1891) in particular has described sense cells near the hairs and skin.

[3] Dawson (1920) has described deep lying sense cells in the musculature of the earthworm which may possibly be regarded as proprioceptors.

renewal of the stimulus elicits an immediate effect. This recovery phenomenon is seen in preparation IB, second walking leg of *Charybdella*, after $1\frac{1}{3}$ hours in sea water, 24 June, temperature 275°C. The weight suspended from the limb was lifted and allowed to fall 120 times, once every half second and each fresh extension produced a discharge of afferent impulses which ceased with the movement of the limb in spite of the tension of the weight but re-appeared at the next fall of the weight. The 120th discharge was as vigorous as the first. This recovery from adaptation is not unlike that found in the vertebrate muscle spindle (Bronk, 1929) but of course the adaptation of the latter is not rapid.[4] The crustacean proprioceptors, like the sensoria associated with tactile hairs in vertebrates (Adrian, 1928) exhibit the primitive condition of rapid adaptation like the nerve fibre itself. The power to respond to a persistent stimulus is a characteristic of the more elaborately evolved vertebrate muscle spindle.

The maintained standing posture of the higher vertebrates is made possible by the persistent tension receptors. Most crustacea, however, when out of water are supported by the ventral surface which rests on the ground when the animal is not moving. The nature of the movement receptors discussed above obviously helps to explain this posture. When in the sea the ventral surface of the body is frequently sustained above the substratum; this is often due to the passive support of the legs, to the buoyancy of the water or to the support afforded by the most posterior pair of legs which are typically resting on a vertical rock surface. The exaggerated standing posture of the male *Callinectes* during courtship is maintained by the active movements of the swimming legs.

The significance of these afferent discharges for the pseudo-reflex movements of the excised limb will appear in the sequel.

Tonus. In a previous paper (Barnes, 1930b) it was reported that peripheral tone in the excised limb of *Cancer pagurus* and the lobster is produced by prolonged discharges of motor impulses from the cut end of the nerve. An oscillograph record was given showing a remarkably regular rythm in a single fibre of the nerve associated with a persistent contraction. In the crustacean nerve-muscle preparation it is a simple matter to obtain records of the action current in a single fibre. Hoffmann (1914) showed that the contraction of a muscle could be produced by a single nerve fibre, in fact the opening muscle of the claw and the extensor muscle moving the preceding segment are supplied with the same excitatory fibre.

[4] Matthews (1931) in a recent paper defines adaptation as the gradual fall in the frequency of the discharge under a constant stimulus. He considers the possibility of the myelin sheath acting as a reservoir for a substance necessary for excitation which diffuses slowly to the discharged membrane. In the unmedullated nerve endings of crustacea, however, the recovery from adaptation is probably a more simple type of diffusion mechanism.

Independent movement of these two muscles is made possible by the inhibitory fibres one of which supplies each effector. In many of the Bermuda preparations discharges lasting as long as 60 seconds were set up in the motor fibres by cutting the nerve at its distal extremity while in place on the electrodes and in confirmation of previous results muscular contractions were observed which corresponded exactly with the nervous discharge. It would appear that all prolonged contraction or tone in these peripheral preparations is a type of tetanus rather than an automatic property of the muscle. This finding is not without significance for the general theory of tonus and has important bearing on the analysis of pseudo-reflexes which is given in the next sections of this paper. When the nerve is cut a second or third time the resulting volley of motor impulses is much weaker and of shorter duration indicating that the motor fibres have not completely recovered. The tendency of crustacean motor fibres to become discharged has been mentioned by Levin (1927) and Furusawa (1929). This property is associated with the fatiguability of these unmedullated fibres and Hill (1929) has called attention to the analogous property of elements in the central nervous system of vertebrates. It is a curious fact (reported by the writer in a former paper (Barnes, 1930a)) that cutting the purely sensory nerve leaving the dactylopodite does not give rise to impulses. In all experimental work with the crustacean nerve muscle preparation it is quite essential to make sure that no motor impulses are arising from the end of the nerve severed during dissection; these impulses can be detected with an amplifier and leading off electrodes.

Responses of isolated limbs associated with muscle and nerve stretching. The foregoing discussion of sensory and motor impulses and their origin has cleared the ground for a consideration of the combination of these two types of nervous discharge occurring in the "reflex" movement of the isolated limb. The writer had previously observed rare instances of "reflex action" in excised Arthropod limbs on three occasions. These curious responses were observed in the claw of *Cancer pagurus* from the North sea in the laboratory of Prof. E. D. Adrian at Cambridge; in the legs of *Lucanus* from the Bois de Boloigne in the laboratory of Prof. L. Lapicque at the Sorbonne; and especially in small Mediterranean spider crabs while the writer was working under the direction of Prof. H. Cardot at the Tamaris Marine Station. The Bermuda preparations, however, exhibited these reactions to a remarkable degree and invited further analysis of the factors involved.

The position of the limb and the leading off electrodes may be seen in figure 1. It will be observed that the limb has no connection with the central nervous system (ventral nerve cord) and that no stimulating electrodes were employed. The latter condition eliminates possible sources of error from current escape. The limb was stimulated by bending or extending various joints which in favorable preparations immediately elicited respon-

sive movements in various directions in spite of the absence of the central link in the reflex arc.

A reflex action not involving the central nervous system has been described by Alexandrowicz (1909) in the sympathetic nervous system of crustacea. In the visceral plexus of *Astacus* there occur bipolar sensory cells sending one off-shoot into the epithelium of the mucosa and another into the muscles of the intestine, thus establishing a peripheral reflex arc. "Axon reflexes" are known in the crustacean limb (cf. Bayliss, 1927): if a claw muscle is stimulated directly by electrical means the impulse may travel back over the motor fibre and follow a branch of this to another muscle causing contraction. The well known property of nerve fibres to conduct in both directions has been diagrammatically demonstrated in the crustacean limb (cf. Buddenbrock, 1928).

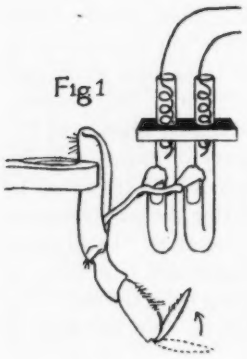

Fig. 1. Limb of *Eupanopeus* with leading off non-polarizable electrodes on the nerve. The terminal segment is responding (by maintained contraction) to a proprioceptive stimulation.

It will be recalled that vaso-dilatation in the vertebrate skin may also involve an axon reflex (Krogh, 1924); here the stimulation of sensory nerve in the skin gives rise to afferent impulses which follow a branch of the sensory fibre leading to a capillary and evoke dilatation. In this case it is not improbable that the stimulating agent is a substance secreted by the sensory nerve fibre (Lewis, 1927).

Very few of these reflexes which do not involve the central nervous system have been reported so that it may not be out of place to consider the "reflex" movements of the Bermuda preparations in some detail. Moreover, these examples are unique for there is no anatomical connection between the receptors and effectors. It is necessary to eliminate every possible kind of manipulation of the excised limb which might produce mechanical or passive movements. Pressure applied to the limb may lead to movements in several ways: 1. When the clamp holding the meropodite of *Goniopsis* is tightened, the extensor muscle is squeezed upwards producing passive extension of the more distal segments. 2. Pinching the meropodite of *Goniopsis* and other forms (when the limb contains blood or sea water) forces the fluid distally; the skin at the joint swells and extension results.[5] In *Goniopsis* effects 2 and 3 may be opposed by gradually increasing the pressure, until the nerve

[5] The "shed" limb of the spiney lobster exhibits this hydrostatic effect in a striking way. If the finger is pressed against the proximal end of the leg, all the segments slowly extend. This was demonstrated by Mr. Giiessen of the Bermuda Station.

is stimulated and a rapid maximal flexion immediately overcomes the hydrostatic extension. This was particularly striking in preparation 29E, leg of *Goniopsis* (nerve in situ), 18 July, temperature 26.2°C., shortly after excision. Preparation 28A of the same species, 17 July, 26.8°C. exhibited rhythmic movements of the extended dactylopodite when the pressure affected the meropodite nerve.

Stimulation by moving the segments may set the motor fibres in action by stretching the nerve. That stretching may serve as a stimulus is readily demonstrated by pulling on a thread tied to the proximal end of the nerve. The effect is easily produced when the preparation is fresh but may sometimes be obtained after an hour or more. In preparation IC, second walking limb of *Carybdella*, extension was elicited in this way after two hours immersion in sea water (24 June, 27.5°C.). The nerve lies rather loosely between the muscles; and flexion of the joints causes visible displacements of the nerve trunk and motor branches running to the muscles. In the large claw of *Cancer* measurements were made at Cambridge of the extent of the movement in the meropodite nerve resulting from movement of the claw. The traction on the nerve may change its broadest diameter to $\frac{2}{3}$ of the resting size and the longitudinal movement of carbon particles on the nerve may reach several millimetres. Further studies of this type of excitation were made with the more favorable Bermuda preparations and the discharge of the nerve was followed with the amplifier.

Preparation 37A, (class of *Callinectes*, after 25 minutes in sea water, 20 July, temperature 27.8°C.) may be selected for analysis. The limb was held in a vertical position, claw downwards by a clamp on the meropodite; the proximal part of the nerve from this segment rested across the electrodes. The preparation was stimulated by moving the propodite or "hand" of the claw in a flexed position and allowing it to fall back to its original posture. This movement exerted traction on the muscles contained in the carpopodite or "wrist" and on the nerve and also set up a brief discharge of proprioceptive impulses. The response, which was immediate, consisted of rhythmic movements of the dactylopodite, 4 mm. in extent which lasted for about 70 seconds with a frequency of 3 flexions every two seconds. The frequency and extent of these movements gradually declined. There was a constant discharge of motor impulses in the nerve which ceased exactly with the muscular movements. Prolonged discharges in motor fibres set up by a single stimulus are discussed above. It seems probable that the stretching of the nerve served as the stimulus leading to the responsive movements. The possibility that the muscles themselves were stimulated directly by stretching may be ruled out for the muscles pulled directly by the stimulating movement (those in the carpopodite which move the propodite) have no connection with the responding digit. The simplicity of the musculature in the crustacean limb permits immediate analysis of

all movements; each segment is moved in one plane only by its single flexor and extensor situated in the next proximal segment.

Stretching the muscles in rare instances evokes a rhythmic response in them and in certain preparations in which the muscles pulled by the stimulating movement are involved in the responding flexion or extension, this factor must be considered especially when no motor impulses in the nerve were detected by the amplifier. Preparation 22A, leg of *Callinectes*, after 2½ hours in sea water, 5 July, 25.5°C., is selected as an example. The terminal segment was moved to a flexed position and fell passively to its original extended posture and exhibited slight rhythmic movements for several seconds. No motor discharges were detected by the amplifier in the nerve during the response. A beautiful preparation can be made to show these rhythmic stretch responses in the isolated muscle. Preparation 10A, flexor from the meropodite of *Callinectes*, 1 July, temperature 23.5°C., will illustrate the point. After removal of the main nerve trunk the flexor was pulled out of the leg and observed in sea water. Here and there the individual strands of muscle were pulsating, the whole preparation resembling a sea anemone with vibrating tentacles (fig. 2). The active strands were pulsating every ⅕ second for over six minutes, the extent of each movement being about 1 millimetre. This slight linear movement is quite significant; the leverage of the dactylopodite is considerable. For example, carbon particles on the extensor muscle in the "hand" move only 0.5 mm. when the digit moves 4 mm.

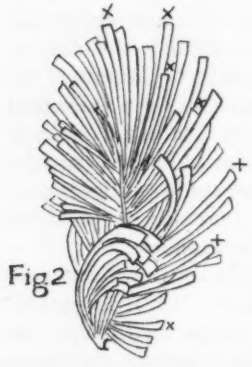

Fig. 2. Flexor muscle of *Callinectes* in which individual strands (marked *X*) have been set pulsating by a stretch stimulus.

Unexplained responses in isolated limbs. The preceding discussion will serve as a preparation for the analysis of a more interesting type of anomalous "reflex" in which there is evidence that the activating principle is not traction on the nerve or on the muscle. Preparation 14A, walking limb of *Eupanopeus*, after 3½ hours in sea water, 2 July, temperature 25°C., will serve as an illustration. The meropodite nerve was on the electrodes and strong sensory impulses were elicited by stimulating the movement receptors and tactile hairs. It was noted that flexion set up a more vigorous proprioceptive discharge than did extension. The best case will be described in some detail. The limb was held vertically and the dactylopodite was moved to a flexed position which caused a discharge of sensory impulses, accompanied by a prolonged discharge of impulses in the motor fibres which maintained the flexor muscle of the dactylopodite in a contracted

state for over 60 seconds. This response of the limb is shown in figure 2. The motor discharge died away when the flexion ceased. It is suggested that the motor fibres were not activated by stretching and for the following reasons—1, the short flexion of the dactylopodite in the walking leg does not disturb the nerve trunk like the more massive movements of the claw discussed above; 2, there are no motor fibres in the dactylopodite. It might be urged that a muscle was stimulated directly by stretching and that the nervous discharge reproduced by the amplifier proceeded exclusively from the movement receptors. There are two considerations which make this suggestion difficult to defend—1, the proprioceptors exhibited very rapid adaptation hence they could not respond for the duration of the contraction, 2, the actual muscle stretched, the extensor did not contract.

The possibility remains that the motor fibre running to the extensor of the dactylopodite was in some way excited by the sensory impulses in the adjacent sensory fibres. It is well known that the e.m.f. of crustacean nerve is very high (Biedermann, 1895; Furusawa, 1929) and it is suggested that the action current in the sensory fibres lying next to the motor fibre provided the stimulus. Two considerations tend to support this hypothesis; 1, the isolated motor fibres are surrounded by hundreds of sensory fibres. In *Cancer pagurus* the writer estimated over ten thousand sensory fibres[6] to six motor fibres in certain locations in the nerve trunk (Cambridge data); 2, both sensory and motor fibres are unmedullated and hence the insulation is poor (cf. Kappers 1929 for a brief discussion of the insulating properties of the myelin sheath). Moreover a large proportion of the sensory fibres can be activated in the dactylopodite which is the chief sensory field of the leg. The nerve bundle of sensory fibres leaving the dactylopodite may be almost half the diameter of the entire nerve trunk in the proximal segments. Furusawa (1929) gives the action current in the entire nerve of *Maia* under electrical stimulation as several millivolts but it must be admitted that the threshold for stimulation in the most rapid and excitable nerve fibres is rather high. Munnier and Dubuisson (1931) found a rheobase of 0.08 volt in these fibres of *Callinectes*. However, it is possible that a motor fibre surrounded by many active sensory fibres might be stimulated under favorable conditions. The writer (Barnes, 1930a) has previously measured the potential changes of individual impulses both motor and sensory which are of the order of 15 to 20 microvolts.

The high threshold for stimulation by electrical means allows another type of influence exerted by the active sensory fibres to be considered, i.e., a chemical one. In recent years the "humoral theory" of nervous action has found support (Cardot, 1929), in fact, the most striking example of

[6] The diameter and receptive fields of these sensory fibres have been briefly described (Barnes, 1930c, 1931) and a more extensive analysis will be published shortly.

the chemical action of a sensory nerve was found by Perkins (1928) in crustacea. The optic nerve of *Palaemonetes* secretes a substance exerting chemical control of the chromatophores. The chemical action of sensory fibres in the vertebrate skin has been mentioned above. In the crustacean preparations the cut ends of the sensory and motor fibres in very close proximity afford the possibility of more direct influence by chemical means. It is known that ammonia, a highly active substance, is produced by nerves (Winterstein, 1925) and the ammonia production increases to a greater extent on activity of the nerve than does the CO_2 evolution. Moreover, the metabolism of crustacean nerve is particularly high (Hill, 1929).

In conclusion it may be said that the actual experimental results checked with the amplifier are obviously more important than the electrical and chemical hypothesis put forward to explain the action of the sensory impulses upon the motor fibre which discharged.

It is clear that the description of animal movements in terms of the "reflex arc" concept is often a matter of difficulty and it is to be hoped that this notion will be ultimately replaced by the more fruitful theory of tropisms as developed by Crozier (1929).

SUMMARY

1. Proprioceptive impulse discharges are set up in crustacean nerve by movements of the limbs. In all cases the "adaptation" to the stimulus is very rapid.

2. Prolonged contractions or tonus in the limb muscles are always associated with corresponding discharges in the motor fibres.

3. The excised crustacean limb will respond in a "reflex" manner to natural stimulation. The nature of these anomalous "reflex" phenomena and the influence of the sensory nerve fibres upon the adjacent motor fibres are discussed.

Parts of the above discussions are based on results secured in the laboratory of Prof. E. D. Adrian at Cambridge but it is obvious that the writer is entirely responsible for the interpretations put forward.

It is a pleasure to acknowledge the encouragement and facilities extended to the writer by Prof. E. G. Conklin at the Bermuda Station.

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ON THE MECHANISM OF OVULATION IN THE RABBIT

V. The Effect of Direct Intrafollicular Injections of Extracts of Urine of Pregnancy

MAURICE H. FRIEDMAN

From the Department of Physiology, University of Pennsylvania Medical School

Received for publication October 6, 1931

In spite of the fact that the biological effects of urine of pregnancy closely resemble the effects obtained with substances extracted from the anterior pituitary, it has not been conclusively demonstrated that the urinary substances are derived from the pituitary. Nor has it been shown that the urinary substances act directly on the ovarian follicles. In the hands of Evans (1931), for example, the extracts of urine of pregnancy have been totally ineffective in hypophysectomised animals, and this worker suggests that the urinary substance acts only through its ability to activate a "prohormone" in the pituitary gland. Hill and Parkes (1931a) also found difficulty in demonstrating the effects of urinary extracts in hypophysectomised animals. When these workers used whole, untreated urine, however, they experienced no difficulty whatsoever in producing ovulation in acutely hypophysectomised animals (1931b). In their opinion, the relative inefficiency of the urinary extracts "was probably accounted for by the high concentration of toxic substances characteristic of many of the extracts." Certainly, this possibility must be seriously considered.

The method of direct intrafollicular injection seemed to offer a means of attack which would obviate the inevitable trauma which is incident to hypophysectomy at the hands of all but the most skilled operators. Moreover, if urinary substances are capable of acting directly on the ovarian follicles, one might expect to secure, by intrafollicular injection, results with smaller quantities of extract than are required for results with intravenous or subcutaneous administration. One might then avoid the necessity of using quantities of extract large enough to contain toxic materials in an amount which could interfere with the results.

Methods. For reasons already given in earlier publications post-partum rabbits were used exclusively in these experiments (Friedman, 1931a, b). The extracts of urine of pregnancy were secured from Parke, Davis & Co., and were prepared from whole urine by a process of precipitation by alcohol. The injections were made with a tuberculin syringe to which was attached a no. 26 hypodermic needle, modified to suit the present purposes by hav-

ing the point filed down so that the length of the bevel was only about one-half its original length.

Under ether anesthesia one ovary was exposed through a flank incision and mobilised ventrally through the wound. The ovary was then surrounded by gauze packs to isolate it from other structures and to prevent any of the material used for injection from leaking into the abdominal cavity. By grasping the ovarian pedicle at each pole of the ovary an assistant held the organ firmly, and by placing his middle finger along the hilum of the organ, he offered a relatively steady support against which to rest the needle. With such support, the needle was cautiously thrust into a large follicle. This always occasioned the escape of some follicular fluid. By gently pressing on the plunger, the ruptured follicle was slowly irrigated with a small quantity of extract, care being exercised to catch the excess extract in the gauze packs. When all the ripe follicles in the one ovary had been irrigated, the gauze was removed, the ovary returned to its normal position, and the flank incision closed in three layers. To irrigate the several follicles in each ovary, the tuberculin syringe was filled beforehand with not more than one and one-half times the calculated minimal effective dose of the extract in question (Friedman, 1931b). In most instances not more than one-half the contents of the syringe was used to irrigate the several follicles. Of course, all operative procedures were conducted under strictly aseptic conditions.

RESULTS. The first eight experiments were performed with an extract of which 0.07 cc. per kilo was required to produce ovulation in a post-partum rabbit (see extract no. 1, Friedman, 1931b). In not one of these eight rabbits did the follicular irrigation result in the growth of lutein tissue.

For the remainder of the experiments a more powerful extract was employed. Only 0.02 cc. per kilo of this extract was required to provoke ovulation in a post-partum rabbit. In eighteen of the twenty-two rabbits in which this extract was used, corpora lutea were grossly visible in the injected ovary after the fourth day following the operation. In seventeen of these rabbits the unoperated ovary showed no change, presenting only clear, unruptured follicles. In one of the eighteen rabbits, however, several recent corpora lutea were found in the unoperated ovary, indicating that the gauze blockade had not been effective. Gross inspection of the injected ovaries of the remaining four of the twenty-two rabbits disclosed no lutein bodies, and a microscopic search of the irrigated follicles in these ovaries revealed no trace of lutein tissue. Similarly, a microscopic examination of the unoperated ovaries in these four animals disclosed no evidence of luteinisation.

Of the seventeen rabbits in which the operation had produced unilateral corpora lutea, five were sacrificed on the fifth day following the injection. In each of these five rabbits from two to six corpora lutea

were found in the injected ovary. These corpora lutea, on gross inspection, compared favorably in size and vascularity with the corpora lutea of the fifth day of pseudopregnancy. Microscopically, however, they differed somewhat in having a hollow center which was partially filled with blood (fig. 1). Histological examination of the unoperated ovaries of each of these five animals disclosed no discernible differences from the picture seen in a normal ovary of a rabbit in heat.

Seven of the seventeen rabbits were subjected to a second operation on the fifth day following the follicular injection. At this time one uterine horn was slit longitudinally at the free border, and several linen sutures passed through the muscularis and mucosa. During this operation it was noted that the injected ovary in each doe contained two or more satisfactory corpora lutea while the unoperated ovary contained only clear, unruptured follicles. Six days later these seven animals were autopsied. By this time the corpora lutea had become pale and decidedly shrunken, so that they were not at all comparable with the corpora lutea of the 11th or 12th day of pseudopregnancy. Under the microscope the artificial corpora lutea were seen to be quite solidly filled with lutein cells. These lutein cells, however, were very much vacuolated, differing markedly in this respect from the lutein cells of the ordinary corpus luteum of the same age. The follicles of the unoperated ovaries in these animals contained no lutein tissue at all. The uterine horns of these seven rabbits showed red, nodular enlargements at the site of the sutures. These proved to be merely nodules of edematous mucosa which were entirely devoid of decidual cells.

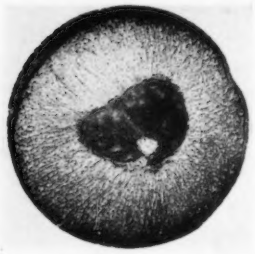

Fig. 1. Microphotograph of a section through a corpus luteum five days after the intrafollicular injection of an extract of urine of pregnancy (x60). Formalin fixation, H. and E. stain.

In three rabbits of this series the uterine horn was threaded at the time of the follicular irrigation. Eight days later the rabbits were sacrificed and their tissues preserved for study. The lutein bodies in the injected ovaries were similar to those of the seven rabbits described above, although the degree of vacuolisation was less marked. As before, the unoperated ovary possessed no lutein tissue. In these rabbits also the nodules at the site of the threads in the uterus consisted only of edematous mucosa.

The remaining two of the seventeen rabbits were placed with a male on the third day following the follicular injection. Both does were very much in heat and willingly accepted coitus. Twenty-four hours later an autopsy of these two females showed good corpora lutea in the injected ovary. These lutein bodies were quite similar in size and ap-

pearance to the ordinary corpora lutea on the fourth day of pseudopregnancy. Yet, the ovary on the unoperated side contained several freshly ruptured follicles, giving adequate evidence of the occurrence of ovulation.

In addition to the thirty experiments in which extracts of urine of pregnancy were employed, six experiments were conducted in which sterile saline was used to inject the follicles in place of the extracts. In no instance did the irrigation with saline result in the appearance of lutein tissue.

Discussion. The results obtained in these experiments clearly show that it is possible to luteinise mature follicles by the direct intrafollicular injection of extracts of urine of pregnancy. The uniform failure to produce luteinisation by the intrafollicular injection of relatively weak extracts, or sterile saline, is sufficient evidence that we are not dealing with some purely mechanical effect, but with an effect attributable to something contained in the extract. The absence of any discernible change in the unoperated ovary indicates that this unilateral luteinisation is effected in the absence of any general humoral change capable of provoking ovulation or luteinisation of unruptured follicles. It is barely possible that follicles subjected to irrigation are more susceptible to small quantities of a luteinising agent than are unruptured follicles. This possibility must be especially considered in view of the finding that considerable amounts of an extract of urine of pregnancy may be injected into a rabbit without producing any visible effect on unruptured follicles (Friedman, 1931b). It would not be safe, therefore, to assume that the unilateral production of corpora lutea in these experiments was due entirely to the direct action of the extracts without the aid of any general humoral change, although this does seem quite probable.

Whatever be the cause of the production of these corpora lutea, their early degeneration, and their apparent functional deficiencies are not easily explained. Perhaps the early degeneration and the apparent functional deficiencies are closely connected. For example, the uniform failure to produce a decidual reaction in the presence of the unilateral corpora lutea may be explained as easily by the early degeneration of the lutein tissue as by any hypothesis based on some peculiar functional deficiency of this lutein tissue during its period of growth. The failure of the unilateral corpora lutea to prevent ovulation from the opposite ovary on the third day after the operation is not so readily accounted for. It is conceivable that the secretions from the unoperated ovary might interfere peripherally with the functional activity of the corpora lutea in the injected ovary. Moreover, it is possible that the untreated ovary interferes with the growth of lutein tissue either by secreting some agent which inhibits lutein development or by competing with the lutein tissue for some essential substance in the blood stream.

In addition to the factors involving the untreated ovary, one must, of course, consider the factor of operative trauma. Still, it is difficult to imagine a kind of operative trauma which would allow a very satisfactory growth of lutein tissue for five or six days and yet interfere with further lutein development.

Some recent work of Smith emphasizes another factor which must be regarded as pertinent to the present discussion. In his experiments on rabbits (1931) in which hypophysectomy was performed a few hours after coitus, ovulation occurred, but the discharged follicles did not develop into normally growing corpora lutea. After a day or two the young corpora lutea showed definite signs of regression. If the hypophysectomy was performed two or three days after ovulation had occurred, the development of the corpora lutea was curtailed a day or two later. Unless one attributes these results to some incidental operative trauma to parts adjacent to the hypophysis, it seems impossible to avoid the conclusion that the presence of an intact pituitary is essential for the normal luteinisation of discharged follicles. It might well be, therefore, that the early degeneration of the unilateral corpora lutea in the present series of experiments was due to failure of our operative procedures to stimulate the hypophysis to furnish adequate quantities of some indispensable hormone.

At the present time experiments have been undertaken with the hope of determining which of the several possible factors is chiefly responsible for the early degeneration of the unilateral corpora lutea.

SUMMARY

1. It is possible to luteinise mature ovarian follicles by the direct intrafollicular injection of extracts of urine of pregnancy.

2. By this means it is possible to produce corpora lutea unilaterally in the absence of general humoral changes of sufficient magnitude to cause any discernible change in the follicles of the opposite, untreated ovary.

3. Corpora lutea produced in this manner suffer regression much earlier than do the corpora lutea of pregnancy or pseudopregnancy.

4. By the means employed in these experiments it has been impossible to demonstrate any functional activity on the part of these unilateral corpora lutea.

This work was aided by a grant from the Committee on Research in Problems on Sex of the National Research Council. I am also greatly indebted to Doctor Bugbee, Doctor Kamm, and Doctor Sharpe of Parke, Davis & Co. for their generous aid.

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HIGH AND LOW FREQUENCY ELECTRICAL RESISTANCE CHANGES IN DYING VOLUNTARY MUSCLE OF RABBITS

ALLAN HEMINGWAY AND DEAN A. COLLINS

From the Department of Physiological Chemistry and the Department of Physiology, The Medical School, University of Minnesota

Received for publication September 28, 1931

Hermann (1872) measured the electrical resistance across and along frog muscle and found that the transverse resistance was approximately seven times the longitudinal resistance. As the muscle died the transverse resistance decreased until it reached a value equal to that of the longitudinal resistance which remained unchanged. Galeotti (1902) observed first an increase in electrical resistance of dogs' muscle on death followed by a decrease. He believed that the final decrease in electrical resistance was due to accumulation of ions resulting from autolysis, a belief shared by Kodis (1901). However, subsequent experiments by Galeotti (1904) showed that there was no increase in the freezing point depression of the tissue to parallel the increased conductivity. McClendon (1910) suggested that the resistance change on death of the muscle might be due to a change in permeability of the cell membranes. Crile, Hosmer and Rowland (1922) measured electrical resistance of voluntary muscle at body temperature and their numerical results indicate a 2 per cent increase in resistance two hours after death.

By using a method whereby the tissue remains intact in situ and maintaining body temperature after death it is possible by taking high and low frequency measurements to determine whether the electrical resistance change is due to a cell membrane effect or to increase in intracellular ionic concentration. We have, therefore, measured the electrical resistance with high and low frequency alternating current at different time intervals before and after death in the experiments reported in this paper.

In the high frequency measurements of Wildemuth (1911) McClendon (1926), (1927), (1929), and Christie (1928) the tissue resistance at high frequencies has been measured, but in connection with other problems and questions as to high frequency technique and application may be referred to these papers.

METHOD. To measure the high and low frequency conductivities the Wheatstone bridge was used which was originally designed by McClendon rebuilt (1929) from a description given by Stratton (1926). The bridge

shielding has been redesigned and built into a compact unit. For high frequencies of one million cycles per second, a heterodyne method of detection was used. For a lower frequency of 1000 cycles per second a telephone and amplifier was used for detection. A Vreeland oscillator generated the alternating current. The bridge is completely shielded and is equipped with a Wagner Ground. Using non-inductive, low distributed capacity, resistance standards the low frequency values check the one million cycle values within one per cent. The accuracy of the 1000 cycle measurements is 0.1 per cent or better.

To hold the living animal for the experiment a double walled constant temperature box was constructed with water of body temperature circulating continuously between the walls. Small openings in the side of this constant temperature box admitted wires to the conductivity electrodes while a circular opening at the end was made large enough so that the head of a rabbit protruded form the box and the body of the rabbit was inside. This permitted the body of the rabbit to be immersed in a constant temperature air bath which is essential for high frequency measurements. Cork, for purposes of heat and electrical insulation, was placed between the wires to the electrode and the circular holes in the sides of the metallic box, while cotton pads and absorbent cotton acted as insulation between the neck of the rabbit and the circular opening in the end of the box. Electrodes consisting of circular plates of block tin 14 mm. in diameter were placed in contact with the musculature of the thigh beneath the skin and fascia. The skin was then sewed together over the electrode except at the attachment of the heavy copper wire to the electrode. A similar electrode was sewed under the skin on the other side of the thigh musculature. The two electrodes were held rigidly at a constant distance apart by means of a bar of bakelite.

From experiments of Kohlrausch, Wien (1896), Warburg (1901) and others it is known that the boundary of a metal and a solution behaves to an alternating electrical current as an impedance consisting by definition of a resistance and a reactance. It is also known that the resistance factor becomes negligible with high frequency current or with platinizing the electrodes. Hence using plain tin electrodes and low frequency (1000 cycles per second) current it is necessary to determine this "electrode" resistance, since a low frequency measurement of the tissue would include both "electrode" resistance and tissue resistance.

The electrode resistance was measured by having one fixed and one movable tin electrode in a cylindrical glass tube of a sufficiently large bore to permit displacement of the movable electrode along the axis of the cylinder. The electrodes were placed at different measured intervals apart and their resistances measured with an alternating current of 1000 cycles per second. The distance between electrodes and the corresponding resist-

ances were then plotted and the resistance where the electrode interval became zero (by extrapolation) gave the electrode resistance. The electrode resistance by this method was 31 ohms.

In order to measure temperature of the tissue during the experiments, copper-constantin thermocouples were used with the variable temperature junction inserted behind the electrode, i.e., between electrode and skin. Hence this junction was at the same electrical potential as one electrode. In order to eliminate unnecessary ground connections, which is very desirable for high frequency measurements, the two thermocouple wires from the junction in the tissues were led into two cups containing mercury in a block of bakelite which was attached to the cork insulation where the electrode wires entered the constant temperature chamber. When a temperature measurement was to be taken the copper and constantin wires to the

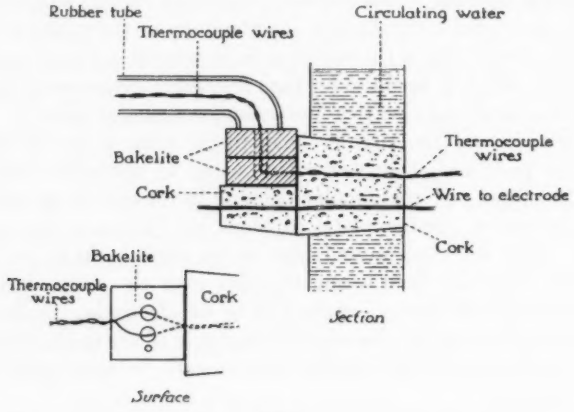

Fig. 1

galvanometer and constant temperature junction, respectively, were connected to the wires to the tissue by a pluglike arrangement shown in figure 1. This was disconnected when a resistance measurement was made.

Experiments consisted of placing a living anesthetized (urethane anesthesia) rabbit with the attached subcutaneous electrodes, within the constant temperature chamber and waiting until equilibrium conditions of constant temperature and resistance were reached. This required from one to four hours. The rabbits were then killed by either 1 bleeding from a cannula previously inserted in the carotid artery, or 2 with an excessive dose of ether. Resistance and temperature measurements were taken at intervals. The living body temperature was maintained after death since all of the rabbit except the head was within the constant temperature air bath.

At high frequency (one million cycles per second) the bridge was balanced by a resistance R in series with a capacity. To balance the bridge at the lower frequency (one thousand cycles per second) the method of McClendon (1926) was used. The same resistance R is placed in series with another variable resistor r and a variable capacity C, in parallel with r. The parallel arrangement of r and C represents the added impedance of the cell membranes and the electrode which becomes effective at the lower frequency but is negligible at the higher frequency. The electrode resistance already obtained is subtracted from the observed resistance r to give a value which is defined as the membrane resistance.

The low frequency capacity reading is a function of the electrode and membrane capacity. Other workers in this field have used the Kohlrausch method of balancing the Wheatstone bridge, which consists simply of a variable resistor in parallel with a variable capacity without the high frequency series resistance. For the purposes of comparison this method of balancing has also been used in our experiments. The resistance measured in this way is designated as the Kohlrausch resistance.

Results. A typical curve of the resistance changes of voluntary muscle on death of the rabbit is given in figure 2. The polarisation and Kohlrausch resistances are corrected for the electrode resistance.

Immediately after the death of the animal the membrane and Kohlrausch (low frequency) resistances show an immediate increase. A maximum is reached in from two to four hours. The maximum value is from 25 to 100 per cent increase over the resistance of the tissue before death of the animal. After the maximum is reached there is a decrease of resistance, at first rapid then more slowly and tending to approach a constant value low in comparison to the living resistance.

The low frequency capacity, which includes both electrode and tissue polarisation capacity, is plotted as a reciprocal value against time. The reciprocal of the low frequency capacity curve parallels the membrane resistance curve.

The high frequency resistance remains practically constant at the value for living tissue for many hours after death and finally shows a slight decrease.

The same changes were observed whether the animal was killed by ether or by hemorrhage. In all ten rabbits were used for the experiments.

Discussion. Gildemeister (1912) has shown that the observed resistance of a tissue decreases with increasing frequency for measurements with audio frequency currents. Phillipson (1920), Fricke (1925) and Cole (1928) have measured the impedance of cells in a suspension at different frequencies and have shown that at high frequencies of one million cycles per second or more, the impedance becomes constant. This constant value has been interpreted as the impedance of a non homogeneous conductor of cells

and intercellular fluid where the cell membranes have no effect as their impedance at these high frequencies is negligible. At low frequencies, however, a resistance measurement would include the resistance of the cell membrane, especially in the case of closely packed cells of muscle or the plant tissues used by Osterhout (1922). Since in muscle the intercellular spaces are relatively small in comparison to the space occupied by the muscle cells, then the high frequency resistance would be practically the resistance of the cell interior. At low frequency a resistance and capacity in

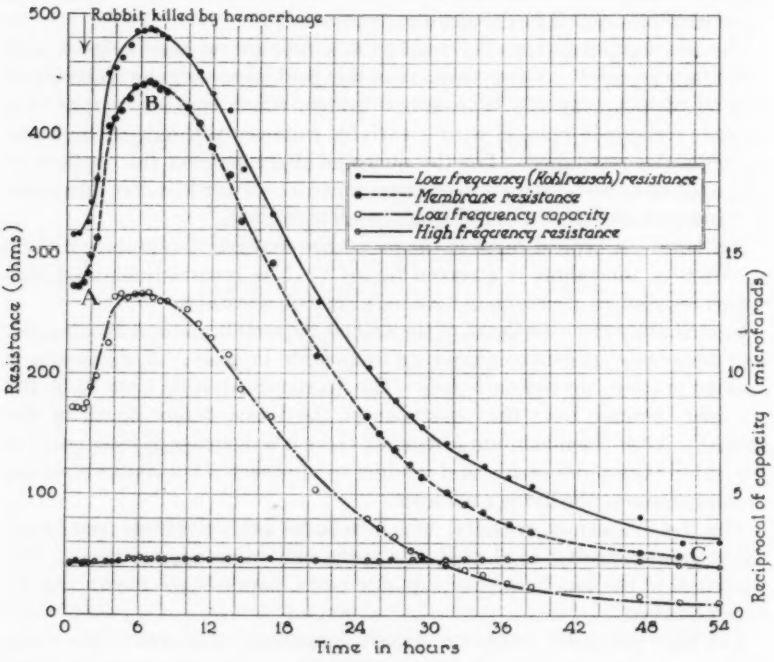

FIG. 2

parallel, in series with the high frequency resistance, would include the resistance and capacity of the electrodes and the resistance and capacity of the cell membranes. The difference then between the low frequency parallel resistance and the electrode resistance would represent the resistance of the cell membranes. The capacity as measured at low frequency would include electrode capacity and the electrical capacity of the cell membranes.

From the curve of figure 2 it is seen that there is apparently a reciprocal relation between membrane resistance and low frequency capacity. If values of membrane resistance are plotted against the reciprocal ($1/C$) of

the low frequency capacity, a straight line results. Two possible theories of autolysis are suggested by this behavior of resistance and capacity. The tissue may be considered as a set of leaky condensers in series, namely, the cell membranes between the low resistant cell interiors. In such a case one condenser considered as a thin plate of area A, thickness d, specific conductivity s and dielectric constant k would have a resistance S and capacity K given by

$$S = d/A \cdot s$$

$$K = kA/4\,\pi\,d$$

whence

$$K = \frac{ks}{4\,\pi}\,\frac{1}{S}$$

If autolysis resulted in a gradual destruction of the cell membranes whereby either 1, the thickness of the cell membranes were decreased, or 2, the number of leaky condensers, i.e., the cell membranes, were decreased in number then the above linear relation between membrane resistance and low frequency capacity would be explained, at least for points on the B C portion of the curve of figure 2.

The high frequency resistance remains practically unchanged while the low frequency resistance undergoes such marked changes. This lends support to the suggestion of McClendon (1910) that the low frequency resistance changes indicate changes within the cell membrane and are not due to an accumulation of ions within the cell as maintained by Kodis (1901) and Galeotti (1902). This is further supported by the observations of Hermann (1872) that there is only a small change of longitudinal d. c. muscle resistance on death but a resistance decrease to one-seventh of the living value when the resistance is measured across the muscle and therefore across the cell membranes. The fact that Galeotti (1904) could find no increase in value of the freezing point depression on death of the muscle is also suggestive. Hence, it may be concluded that resistance changes within dying muscle are due to a resistance change of the cell membranes while the ionic concentration of the cell interior undergoes no appreciable change.

In conclusion we wish to thank Dr. J. F. McClendon of the Department of Physiological Chemistry and Dr. W. K. Stenstrom of the Department of Biophysics for their suggestions, advice and helpful criticism during the course of our experiments.

SUMMARY

The electrical resistance of voluntary muscle of the rabbit has been measured at intervals before and after death of the rabbit. After death of the

animal body temperature has been maintained by a constant temperature air bath. Resistance measurements were made with high frequency alternating current of one million cycles per second and low frequency current of one thousand cycles per second. On death of the animal the high frequency resistance remained unchanged for at least 36 hours while the low frequency resistance at first increased to a value from 25 to 100 per cent of the living value in from 2 to 10 hours, and then steadily decreased. The resistance and capacity measurements made with low frequency resistance indicate a progressive cell membrane destruction as autolysis takes place.

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THE NON-ESSENTIALITY OF THE POSTERIOR HYPOPHYSIS IN PARTURITION[1]

PHILIP E. SMITH

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University

Received for publication September 3, 1931

The effectiveness of extracts of the posterior hypophysis in strengthening uterine contractions at parturition has made it seem probable that the posterior lobe plays an important or even essential part in the initiation and completion of this process. Experimental proof of the liberation of unusual amounts of the oxytocic principle of this lobe, especially into the cerebro-spinal fluid at the termination of pregnancy has been claimed by a number of investigators and denied by others.[2] In 1924 Dixon and Marshall, apparently largely because of Dixon's report (1923) that the C.S.F. contained more of the oxytocic substance at term than at other periods, advanced the hypothesis that the ovaries at this time secreted some substance which activated the posterior hypophysis causing it to liberate a uterine-contracting principle thus inducing parturition. They recognized, however, that the birth of young would take place after ovariectomy and therefore postulated that in the absence of the ovaries some other organ secreted this substance which activated the posterior hypophysis. This hypothesis has been reaffirmed by Marshall (1927). It has been criticised by Vincent (1927), Parkes (1929) and Asdell (1931), the latter admitting the probability of an increase in the oxytocic substance of the C.S.F. at the termination of pregnancy, and by Blau and Hancher (1926), because of the non-specificity of ovarian extracts in stimulating the secretion of an oxytocic substance. Van Dyke (1929) and co-workers believe that calcium is the oxytocic substance of the C.S.F.

An instance has been reported by Dott (1918) in which parturition occurred normally in dogs in the absence of the posterior hypophysis, thus

[1] Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

[2] The literature upon the presence of the oxytocic principle of the posterior hypophysis in the cerebrospinal fluid is extensive and contradictory. Van Dyke and Bailey (1928) have presented excellent evidence that the oxytocic activity of this fluid is due not to a hormone of the posterior hypophysis but to calcium. The literature upon the subject is well reviewed in their paper and by earlier investigators.

showing that in this form the uterus was capable of undergoing the necessary expulsive movements without the secretion of this lobe. To this case I wish to add the findings obtained from a number of hypophysectomies in rats in which, after ablation of the posterior lobe, birth of young took place at the normal time. The complete absence of any fragment of either the intermediate or neural components of the posterior lobe was verified by a microscopic examination of serial sections of the pituitary capsule and its contents.

The parapharyngeal approach (Smith, 1927, 1930) was used in removing the posterior hypophysis. In place of the usual sized cannula by which both the anterior and posterior lobes are readily removed there was substituted a considerably smaller one having an opening of one millimeter or less. After exposure of the hypophysis, the cannula was gently forced through the thin layer of anterior lobe tissue which covers the posterior lobe ventrally, until it was thought that the latter lobe was reached. Negative pressure was then applied. Usually the posterior lobe was either sucked into the cannula or was held by the suction tightly adherent to its end and could thus be withdrawn. In all the cases some anterior lobe was also removed. This did not interfere with sex cycles and gestation since these are normal in rate even after as much as two-thirds of the anterior lobe has been ablated (Smith, 1931). The pituitary stalk and pars tuberalis of the hypophysis were left intact. Following the operation the usual data in regard to the reproductive cycles and body weight were taken for a period of some weeks. Twelve female rats (pied) were found by later serial sectioning of the contents of the hypophyseal capsule to have had all of the posterior lobe removed. Three of these, however, did not show regular sex cycles and at autopsy it was found that there was less than one-third of the normal amount of anterior hypophysis tissue present. That the irregularity of the reproductive cycles in these three animals was not due to absence of the posterior lobe is evidenced, *1*, by the regularity of the cycles in other operated animals which had no posterior lobe but which had larger amounts of anterior hypophyseal tissue, and *2*, by the irregularity or even absence of cycles in animals which had posterior lobe tissue but in which less than one-third the normal amount of anterior lobe was present. The evidence obtained from these animals is thus harmonious with that obtained from posterior-pituitary administration and shows that this lobe is not responsible for the maintenance of the reproductive cycles. Of the remaining nine of the twelve rats which were later found at autopsy to have no posterior hypophysis, six were mated and bore litters, parturition taking place at the normal time. The number of young born in each of these litters was: 6, 8, 7, 6, 3, and one of unknown number, the young being eaten soon after birth. This last animal, however, was mated a second time and had 7

young. In each case the birth took place at the usual time—the twenty-first to the twenty-second day of pregnancy.[3]

Lactation, as evidenced by the growth of the young, also appeared to be normal although the evidence upon this point is not as crucial as is desirable. Three of the litters were removed from the mothers and killed shortly after birth because the rearing of the young would have interfered with other studies for which the animals were being used. In the three other cases the young were reared by the mother and were weaned according to our routine on the twenty-second day of life. The weaning weights of one of these litters was up to the usual weights for our colony, varying from 44 to 50 grams, one was somewhat low, 33 to 41 grams, while one was much below, being but 30 to 33 grams. Since the weaning weights of one of the litters was normal it seems that the low weights of the other litters is probably not due to the absence of the posterior hypophysis. Mothers who do not rear young of a weight satisfactory to us are not uncommon in our colony and are always discarded. The evidence thus indicates that the secretion of the posterior hypophysis is not indispensable for lactation.

The results obtained clearly show that in rats parturition is not prevented by the removal of the posterior hypophysis nor is the period of gestation lengthened. Consequently the secretion of this lobe is not necessary either for the genesis or the maintenance of the birth contractions of the uterus. It is possible that an oxytocic substance may be secreted by other structures at this time. The pituitary stalk is known to contain this principle (Atwell and Marinus, 1918). The minute size of this structure, however, makes it improbable that it could secrete effective amounts of the oxytocic principle. Another possible source of an oxytocic hormone is the hypothalamus. Trendelberg and Sato (1928) have shown that the region of the brain in dogs after hypophysectomy contains more than the normal amounts of a uterine-contracting substance. The experiments reported here do not disprove that some other structure does not supply an oxytocic substance which aids in parturition. They show only that the secretion of the posterior hypophysis, itself, is not essential to parturition.

SUMMARY

The posterior hypophysis was removed in adult female rats and the effect upon parturition noted. The completeness of the ablation was verified by a microscopic examination of serial sections of the pituitary capsule and its contents. In all cases some anterior lobe tissue was also removed but a

[3] It may be of value to note that one of three male rats in which all of the posterior hypophysis was removed, was twice placed with a female in oestrus and mated each time.

sufficient amount remained to maintain normal body growth and sex cycles. Six of these animals were mated and bore litters. Birth occurred at the usual time. The evidence thus shows that in rats a secretion of the posterior hypophysis is unnecessary either for the genesis or the maintenance of the uterine contractions at parturition.

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THE COMPARATIVE SENSITIVITY OF THE REPRODUCTIVE TRACTS OF HYPOPHYSECTOMIZED AND OVARIECTOMIZED RATS TO FOLLICULAR HORMONE[1]

PHILIP E. SMITH

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University

Received for publication September 3, 1931

Experimental work has shown that the follicular[2] hormone acts directly upon the uterus and vagina, whereas the anterior-pituitary sex hormone (or hormones) stimulates the growth, maturation and luteinization of the follicle. It is upon this interrelationship that tests for these hormones are based. The regression of the uterus and vagina which is induced by hypophysectomy is due, if this concept is correct, not directly to the removal of the pituitary, but indirectly to the ovarian atrophy which the pituitary ablation has induced. From this interrelationship it would seem that the uterus and vagina should be as sensitive to the injections of follicular hormone in hypophysectomized as in ovariectomized rodents. Some observations, however, made it questionable if such would be the case. Following hypophysectomy, in addition to the specific atrophy of certain organs (gonads, thyroids, adrenal cortex) all elements of the body fail to undergo normal growth in contrast to the normal size increase occurring after ovariectomy. This is evidenced in the genital system by the postponement, and at times even eventual failure of the vaginal orifice to become established in rats, hypophysectomized before maturity (Smith, 1929), whereas the opening of the vagina takes place at the normal time after ovariectomy (Long and Evans, 1922). There seems to be superimposed upon the vaginal atrophy induced by the absence of the ovarian hormones a direct effect of the pituitary ablation. The findings of Hartman, Geiling and Firor (1930) that the injection of follicular hormone does not cause menstruation in in monkeys after hypophysectomy, whereas similar treatment is effective after ovariectomy (Allen, 1927, 1928; Maddux, 1930; Morley et al., 1930;

[1] Aided by a Grant from the Committee for Research in Problems of Sex of the National Research Council. Thanks are also given to Parke, Davis & Co., and to E. R. Squibb & Sons for contributing part of the extracts used in these experiments.

[2] In lieu of a term which seems more appropriate, I use "follicular" hormone for that principle, whether extracted from ovaries, placentae or the urine of pregnancy, which induces cornification of the vagina in ovariectomized rats and mice.

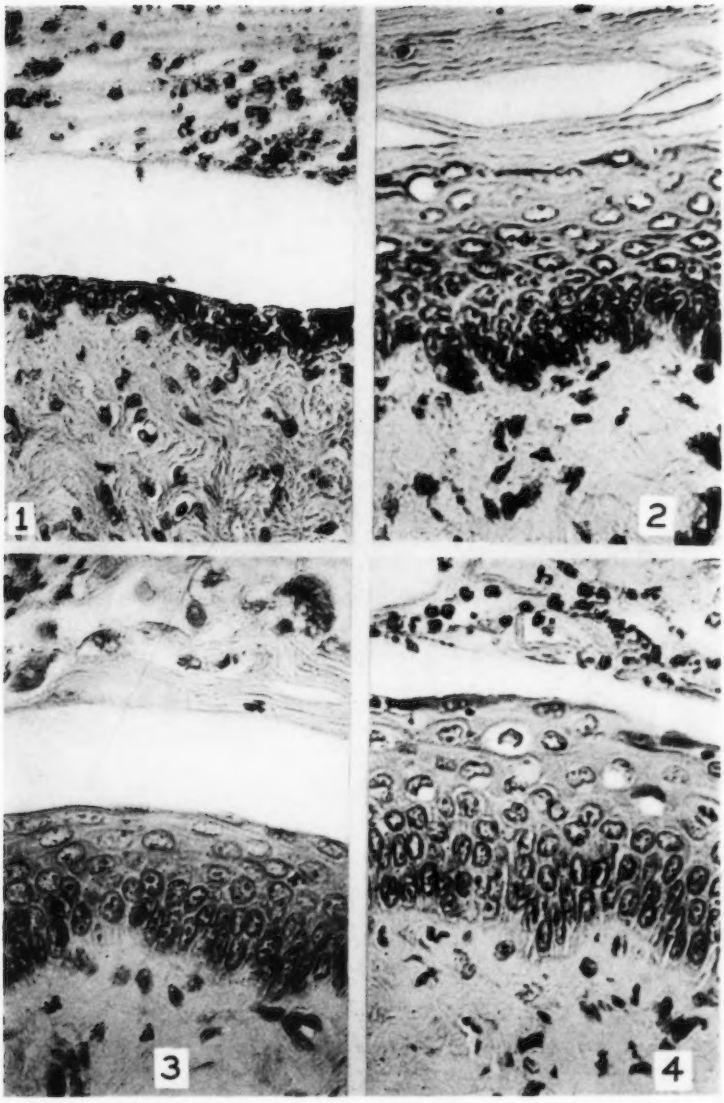
Hartman et al., 1930) also suggest that the response of the reproductive tract to follicular hormone injections might be less in hypophysectomized than in ovariectomized animals.[3]

In order to secure more evidence upon the general problem of the interrelationship of the anterior pituitary, the follicle and the reproductive tract, a series of follicular-hormone injections has been made in rats, half of which were hypophysectomized, the others being ovariectomized. To a part of each of these two types of operated animals a unit dosage of follicular hormone was given without regard to body weight, in others the dosage was proportionate to body weight.[4] More than a single rat unit was given in every case since in some preliminary experiments vaginal cornification was not secured with a single unit. In order to test the mating reactions in the two types of animals massive doses were given in several cases, it having been shown by Marrian and Parkes (1930) that at least 200 times more extract is required to secure mating than is necessary to induce vaginal cornification. Since the extracts were aqueous, the injections were given, as recommended by Bugbee and Simond (1928), over a period of two days, four injections being made each day. Amniotin (Squibb & Son) and Estrogen (Parke, Davis & Co.) were used for injection.

In all, twelve hypophysectomized and an equal number of ovariectomized rats were used. To most of the animals more than one treatment was given, this repeated usage of the animals bringing the total number of treatments to thirty-three for each of the types of operated animals. The treatments were given so that the time after operation and also between

[3] The findings of Hartman *et al.* have an important bearing upon the question of a direct anterior pituitary-uterine (and vaginal) interrelationship, a question with which the present paper is directly concerned. Their studies led them to state that a pituitary secretion was the immediate cause of menstruation, thus giving a direct effect of this gland upon the uterus. Since menstruation has no counterpart in rodents, it seems unwise, until more data are at hand, to make any comparison of the results obtained by them with those given in this paper.

[4] Although the heavier animals of a litter were always hypophysectomized, nevertheless, due to the cessation of growth after pituitary ablation there was always a very considerable difference in weight between the hypophysectomized and ovariectomized rats at the time when treatment was begun. Since the work upon the correlation of body weight to dosage is disharmonious, it seemed wise in some of the tests to give a unit dosage per animal, in others a unit dosage per unit of body weight. Bugbee and Simons (1928) report that the relationship of dosage to body weight is proportionate and have given a formula for determining the dosage of rats of different weights. On the other hand, Laqueur (1927) reports that the weight-dosage relationship is not a linear one. D'Amour and Gustavson (1930) found that the weight factor is of some but no great importance, and Coward and Burn (1927) report no correlation between body weight and dosage. The latter investigators, especially, emphasize the great variability in sensitiveness obtaining between different individuals and even in a single individual at different times.



Photomicrographs showing the vaginal epithelium and underlying connective tissue and the cell types in the vagina. × 480.

Fig. 1. Untreated hypophysectomized rat, G 2900.

Fig. 2. Hypophysectomized rat, G 5462, injected with 3 units of amniotin. Autopsied on the fourth day after the first injection.

Fig. 3. G 5465, an ovariectomized littermate of G 5462, receiving 4½ units of amniotin. The time elapsing in the two animals was identical.

Fig. 4. An unoperated rat, GH 5427, which received 231 units of amniotin. Autopsied on the fourth day.

TABLE 1

Giving the type of vaginal smears and the uterine weights (drained of fluid) of part of the rats injected with follicular hormone

DESIGNATION OF RAT	OPERATION	AGE AT OPERATION	INJECTIONS				WEIGHT OF RAT	TYPES OF VAGINAL SMEAR*				WEIGHT OF UTERUS, 4TH DAY
			Days after		Extract	Total units		3rd day		4th day		
			Operation	Previous injection				9 a.m.	5 p.m.	9 a.m.	5 p.m.	
							grams					
G 5461	Hypophysectomized	124	21	0	Amniotin	2	168	V	V–I	V–II	IV	0.133
G 5462	Hypophysectomized	124	21	0	Amniotin	2	160	V	V	V–II	IV	
GH5464	Ovariectomized	124	21	0	Amniotin	2	206	V	V	V–I	IV	0.066
G 5463	Hypophysectomized	124	21	0	Amniotin	100	179	V	I–II	II	III	
GH5465	Ovariectomized	124	21	0	Amniotin	100	234	V	I–II	II	III	
G 5462	Hypophysectomized		51	30	Amniotin	3	147	V	V	V–II	IV	
G 5463	Hypophysectomized		51	30	Amniotin	3	148	V	V–I	II	I–III	
G 5465	Ovariectomized		51	30	Amniotin	4.6	236	V	V–I	V–II	IV	
G 5462	Hypophysectomized		66	15	Amniotin	3	138	V	V–I	V–II	IV	0.104
G 5463	Hypophysectomized		66	15	Amniotin	3	142	V	V–I	V–II	IV	
G 5465	Ovariectomized		66	15	Amniotin	4.5	240	V	V–I	V–II	IV	0.087
G 5495	Hypophysectomized	118	25	0	Amniotin	3	170	V–I	V–II	II	IV	
G 5493	Ovariectomized	118	25	0	Amniotin	3	210	V	V	V–II	IV	
G 5497	Hypophysectomized	118	25	0	Amniotin	200	185	I–II	II	III	III	0.141
G 5494	Ovariectomized	118	25	0	Amniotin	200	226	I	I–II	II	III–IV	0.140
G 5495	Hypophysectomized		52	27	Amniotin	256	170	V–II	II	III		0.268
G 5493	Ovariectomized		52	27	Amniotin	357	241	V–II	II	III		0.124

W 3944	Hypophysectomized†	194	97	62	Estrogen	12.5	196	V–I	I–II	II–III		0.197
W 3942	Ovariectomized†	194	97	62	Estrogen	12.5	305	V–I	I–II	III–IV		0.144
GH3955	Hypophysectomized‡	191	95	62	Estrogen	12.5	179	V–I	V–II	II–III		0.134
GH3954	Ovariectomized‡	191	95	62	Estrogen	12.5	244	V–I	V–II	III–IV		0.120
GH5429	Hypophysectomized	83	98	0	Amniotin	160	179	II	II–III	III		0.152
GH5427	Normal			0	Amniotin	231	234	II	II–III	III		0.438

* The type of vaginal smear is indicated according to the terminology of Long and Evans (1922). Their stage V (dioestral) in which polymorphonuclear leucocytes predominate is succeeded by a short pro-oestrus (stage I) in which the leucocytes have dropped out and nucleated epithelial cells have increased giving a pure smear of the latter cells. Cornified cells then make their appearance giving a mixture of the two cell types (stage I–II) following which the epithelial cells disappear, cornified cells only being present (stage II)—the oestral stage. These cells increase in number (stage III) following which leucocytes and epithelial cells again appear and the cornified cells decrease in number giving a variable mixture of the three cell types (stage IV). This process continuing leads to the typical dioestral or stage V. With inadequate dosage stages II and III may not appear, there always being some leucocytes and epithelial cells present.

† Received 3 treatments prior to the one reported here.

‡ Received 4 treatments prior to the one reported here.

successive treatments was identical for hypophysectomized and ovariectomized littermates. Autopsies were performed on the fourth day after the first injection. Autopsies were done at this time rather than when the response is maximum (on the third day) in order to secure a longer period for the examination of the vagina.

The comparative effectiveness of the treatment upon the two types of rats was harmonious throughout the entire series and it is consequently thought unnecessary to present the data of all the experiments.

As seen from the table, the vaginal cell types show that the response of the hypophysectomized rats to the follicular hormone injections was fully equal to that of the ovariectomized. Indeed, even in the series in which the dosage given was in proportion to body weight the change to a pro-oestral smear almost invariably came somewhat earlier in the pituitaryless rats. It was fully as pronounced in them as in the ovariectomized littermates. Moreover, it usually persisted somewhat longer, which from the work of Brouha and Simmonet (1927) in immature animals probably indicates a greater sensitivity. The vagina, therefore, is fully as sensitive to stimulation by follicular hormone injections in hypophysectomized as in ovariectomized rats.

A study of serial sections confirms the findings obtained by the vaginal smear method. The vaginal epithelium of rats which have been hypophysectomized undergoes a marked regression (fig. 1). It is thin, being but two or three cell layers in thickness. The cells themselves are small and frequently show pycnotic nuclei. Structurally it is nearly identical with the vaginal epithelium of ovariectomized rats, as shown in Allen's (1924) figure 8. After the injection of even small amounts of follicular hormone, this epithelium becomes several layers in thickness with normal cell types and after dosages adequate to bring on a full oestral type of smear it has a structure nearly identical with that of the normal animal in oestrus (fig. 2). The epithelial growth was as great as in ovariectomized rats given the same dosage (fig. 3).

The uteri of the injected hypophysectomized rats weighed usually more than those of their ovariectomized littermates given the same treatment. Comparison with normals shows, however, that the uterus in the treated operated rats has not attained the usual dioestral weight. Its weight (GH 5429) is much less than in normal rats after injection (GH 5427) or in spontaneous oestrus

A comparison with hypophysectomized rats which have not been injected (table 1, Smith, 1930) reveals that the follicular injections have increased the weight of the uteri. Such was the case even where the dosage was not adequate to induce a complete oestral type of vaginal smear. This is not unexpected since in immature animals the uterus shows a response to oestrin even when the vagina does not (Brouha and Simmonet, 1927). With the

heavier dosages, there was a considerable accumulation of intrauterine fluid, showing that secretion was stimulated. The weight of these uteri is as great as in hypophysectomized rats which had been given daily pituitary implants for a period of two or three weeks (table 4, Smith, 1930).

The reproductive tract of the hypophysectomized rat retains for long periods the capacity to respond to the injection of follicular hormone. In one case (GH 5429) over three months had elapsed between the ablation of the pituitary and the injection of follicular hormone, yet a good response was given.

Attempts with both types of animals were made to secure matings when dosages of 100 R. U., or more, of follicular hormone had been injected. In no case did a mating take place. It seemed probable that the hypophysectomized rats would mate less readily than the ovariectomized ones since they are much less active. It is disappointing that these tests gave no positive differences between the two types. That matings were not secured even with the ovariectomized rats is rather surprising.

The injection of follicular hormone into hypophysectomized and ovariectomized rats thus shows no differences in the response of the uteri or vaginae of the two types to these treatments. After ablation of the hypophysis the response was fully as great as after removal of the ovaries as regards the free cells and structure of the vagina and the weight and structural response of the uterus. This result was unexpected as it was anticipated that the general and profound growth inhibition which results from extirpation of the anterior hypophysis would increase the difficulty of building up the reproductive tract with follicular hormone. The results, however, are harmonious with other types of experiments in rodents which have uniformly failed to show any direct uterine or vaginal interrelationship with the hypophysis and have indicated that any effect which the pituitary has upon the reproductive tract is through the intermediary of the gonads.

SUMMARY

The comparative sensitivity of the uteri and vaginae of hypophysectomized and of ovariectomized rats to injections of follicular hormone has been studied. No essential difference has been found. The response of the vagina as regards the free cell types and the structure of its epithelium and of the uterus as regards its weight and structure was always as great and not infrequently greater in animals which were hypophysectomized than it was in those which were ovariectomized. These findings are harmonious with other experimental work in rodents, which has failed to show any direct interrelationship of the vagina or uterus with the anterior hypophysis.

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A QUANTITATIVE STUDY RELATING THE MAGNITUDE OF PULMONARY VENTILATION TO IMPAIRMENT OF OXIDATIONS PRODUCED BY INTRAVENOUS INJECTION OF ACID AND OF SODIUM CYANIDE

MARIE PELECOVICH

From the Department of Physiology, University of Michigan, Ann Arbor, Michigan

Received for publication September 1, 1931

Recent observations of Gesell and his co-workers (1930) indicated that the increase in hydrogen ion concentration, produced by the intravenous injection of hydrochloric acid, was more predominant in augmenting ventilation than the accompanying impaired oxidations. It was proposed by the authors that further more precise experiments be carried out to study the relation of hyperventilation to impaired oxidations as produced by administration of sodium cyanide and of acids. In accordance with this suggestion, twenty-seven experiments were performed in which twenty-five injections of sodium cyanide, thirty injections of hydrochloric acid, and eleven injections of lactic acid were made. The relation of hyperventilation to the rate of oxygen consumption was quantitatively compared.

PROCEDURE. Dogs weighing 8 to 20 kilograms, anesthetized with urethane and morphine were used. Tidal air, total pulmonary ventilation, blood pressure, and time were recorded. The trachea, external jugular vein, and the common carotid artery were exposed. The trachea was connected with a rebreathing apparatus by means of which pure oxygen was continuously rebreathed. Carbon dioxide was absorbed by a cartridge of soda lime. Oxygen consumption was determined by measuring directly from the record the fall of the calibrated spirometer. The volume of pulmonary ventilation was obtained by an automatically recording gas meter attached to the inspiratory tube of the rebreathing apparatus.

Sodium cyanide (0.003M and 0.005M), hydrochloric acid (0.25M and 0.50M), and lactic acid (0.33M and 0.50M) were injected into the external jugular vein at a uniform rate, according to a method described by Gesell, Krueger, Nicholson, Brassfield and Pelecovich (in preparation for the press). Molar equivalents of sodium bicarbonate were injected after the hydrochloric acid injections, and $\frac{3}{4}$ molar equivalents were injected after lactic acid injections in all instances except where otherwise indicated. Injections were carried to the point where the accessory muscles of respiration came into play, except in cases where the condition of the animal compelled a shorter duration of injection.

It is convenient to divide the twenty-seven experiments into four groups according to the procedure employed.

In the first group of eight experiments, hydrochloric acid was the first injection followed by sodium bicarbonate and then by sodium cyanide. In several experiments, a second injection of hydrochloric acid was made. In order to obviate the possibility of hydrochloric acid influencing the response of the animal to the subsequent injection of sodium cyanide, the above procedure was reversed in a second group of eight experiments in which sodium cyanide was made the first injection.

As it was desirable to eliminate in so far as possible the irregularities of pulmonary ventilation of reflex origin, a third group of four experiments was performed in which the vagi were sectioned. In other respects, the procedure in this series was the same as that in the first.

Finally a fourth group of seven experiments, in which lactic acid and hydrochloric acid were injected was performed.

Oxygen consumption in cubic centimeters per kilogram hour was plotted on the abscissae against pulmonary ventilation in liters per kilogram hour on the ordinates.

Results. Figure 1 represents a typical response to hydrochloric acid and sodium cyanide in the experiments of the first three groups. The numbers in block print express durations of measurements and blood pressure, the latter beneath the former; the figures enclosed in circles indicate the number of the respective determinations.

One hundred sixty-eight cubic centimeters of 0.50M hydrochloric acid were injected over a period of thirty minutes. Eighty-four cubic centimeters of M sodium bicarbonate were then injected over eleven minutes and the animal allowed to rest for thirty-one minutes. Seventy cubic centimeters of 0.005M sodium cyanide were then injected over twenty-six minutes and the animal allowed to rest again for thirty-seven minutes. A second injection of 135 cc. of 0.50M hydrochloric acid was made over a period of twenty-one minutes and this was followed by 67 cc. of M sodium bicarbonate over seven minutes.

Determinations *1* and *2* show that ventilation and oxygen consumption are approximately constant. With hydrochloric acid injection at the end of *2*, oxygen consumption diminishes from 457 cc. to 429 cc. while ventilation increases from 8,768 cc. to 11,590 cc. At *4*, there is a further reduction in oxidation to 418 cc. and an increase in ventilation to 13,035 cc. At *5*, there is a reduction in oxidation to 410 cc. and an increase in ventilation to 16,469 cc., representing an impairment in oxidation below that in *4* of only 10 cc. and associated with an increase in ventilation of 3,434 cc. This relation is more pronounced at *6*, where oxygen consumption falls to 384 cc. while ventilation increases to 32,726 cc. In this case, an impairment of 25 cc. in oxidation is associated with an increase in ventilation of 16,257 cc.

At the end of *6*, hydrochloric acid injection was stopped. The break in the line between *6* and *7* indicates an omission in measurement of oxygen consumption due to an inability to establish a gradient on account of a change in the position of the animal's chest. At *7* sodium bicarbonate was injected. Ventilation drops rapidly to 13,461 cc. while oxygen consumption increases slightly to 388 cc. It is the rule that during sodium bicarbonate administration ventilations drop quickly to nearly the initial level, while oxidations are relatively little changed and thus are considerably below the initial rate. During *8*, *9*, and *10* the ventilations and oxidations remain approximately constant. The former average slightly below the initial level while the latter are decidedly below, averaging 381 cc. as compared with 455 cc.

At the end of *10*, sodium cyanide is injected. There is an initial increase in oxidation to 416 cc. accompanied by an increase in ventilation to 11,807 cc. This unexpected effect has been observed in nineteen out of twenty-five sodium cyanide injections. At *12*, the oxygen consumption falls to 340 cc. and is associated with an increase in ventilation to 14,984 cc. At *13*, oxygen consumption falls to 340 cc. and there is a rise in ventilation to 18,558 cc. At *14* and *15* a slight depression in ventilation occurs accompanied by a fall in oxygen consumption to 256 cc. and 240 cc. respectively. At the end of *15*, sodium cyanide injection is stopped. Oxidation and ventilation continue to fall so that at *16* oxygen consumption is only 164 cc. and ventilation is 15,730 cc. At *17*, there is a large increase in oxidations to 332 cc. with a comparatively small increase in ventilation to 20,766 cc. At *18*, the ventilation falls to 19,469 cc. while oxygen consumption further increases to 397 cc. At *19*, there is an abrupt drop in ventilation to 14,375 cc. accompanied by a slight increase in oxidation to 405 cc. These effects just indicated have been observed a number of times in other experiments with the exception of the immediate effects of cessation of injection represented in *16* of figure 1. The more common immediate effect of cessation of injection was comparatively little change in oxygen consumption and an abrupt fall in ventilation.

At the end of *20*, a second injection of hydrochloric acid is made. There is an initial increase in oxidation accompanied by an increase in ventilation. This initial increase in ventilation and oxygen consumption has been observed in other experiments, although not as constantly as in the case of sodium cyanide injections. At *22*, there is a diminution in oxidation to 379 cc. and an increase in ventilation to 19,602 cc. At *23* and *24* the ventilations rise to 25,751 cc. and 33, 490 cc., while oxidations fall to 333 cc. and 313 cc. respectively. After hydrochloric acid injection is stopped, ventilation falls and oxidation increases as shown in *25*, and this effect is continued when sodium bicarbonate is injected at *26*. Gross inspection of the three "injection curves" shows that there is a vertical tendency to the hydro-

chloric acid curves in contrast to the relatively horizontal tendency to the sodium cyanide curve.

It may be mentioned at this point that the tidal air tracings show a very characteristic irregularity during the sodium cyanide injections. The animal takes occasional deep breaths which become deeper and more frequent as injection progresses. In the hydrochloric acid injections, the ventilation is regular throughout the course of injection. Not infrequently irreg-

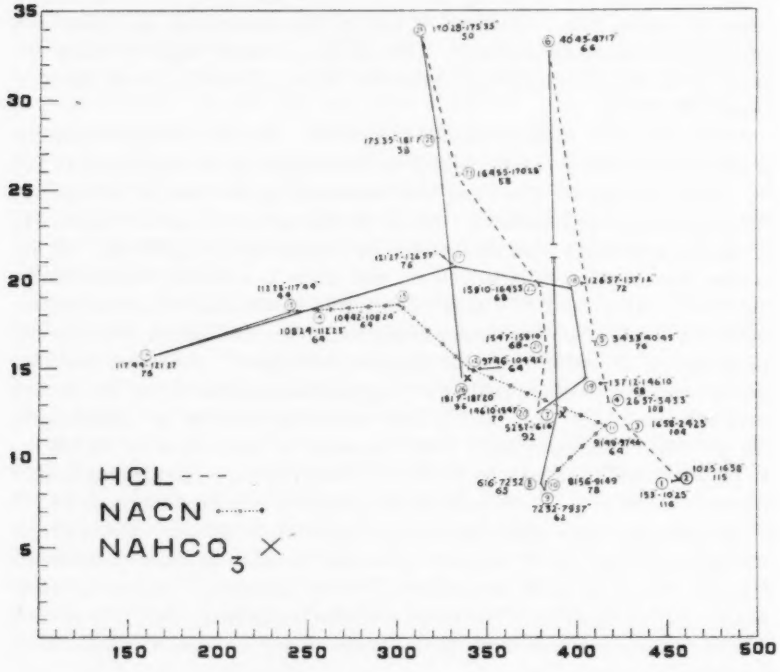

Fig. 1

ular ventilation becomes regular as a result of injection of hydrochloric acid.

SUMMARY OF REMAINING EXPERIMENTS. *Group 1. HCl, NaHCO3 NaCN. Vagi intact.* The hydrochloric acid and sodium cyanide curves in five out of eight experiments in this group resemble with minor variations the curves just described. In two experiments, the sodium cyanide curve parallels the hydrochloric acid curve, i.e., there is about the same amount of increase in ventilation per unit of impaired oxidation with sodium cyanide as with hydrochloric acid. However, the impairment in oxidations pro-

gresses at a lower level than in hydrochloric acid thus placing the curve to the left of the hydrochloric acid curve. Because the animal's condition was poor in the remaining experiment an insufficient amount of hydrochloric acid was injected and no effects were produced. The sodium cyanide effects were similar to those of figure 1.

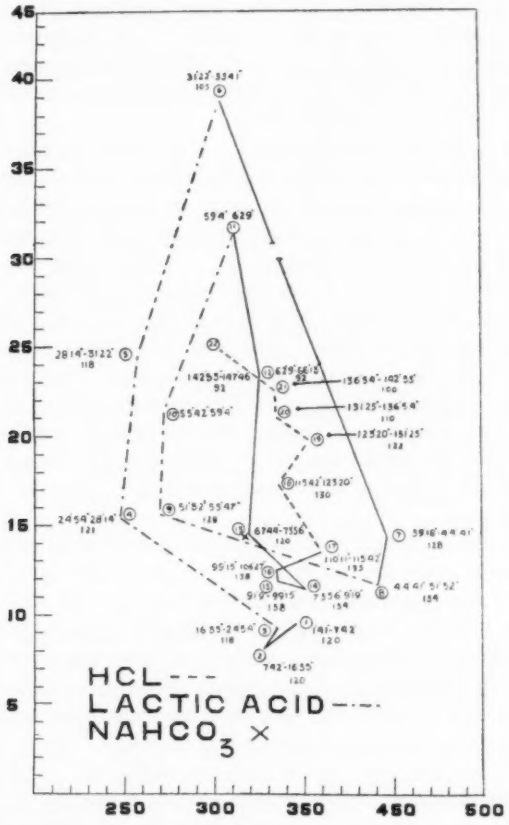

Fig. 2

Group 2. NaCN, HCl. Vagi intact. As was to be expected the condition of the animal was not as favorable after the first injection of sodium cyanide for subsequent experimentation, as it was in the first group where hydrochloric acid was the first injection. Despite this fact, in the majority of experiments, the general character of the curves corresponds to that in

figure 1. The ventilation is never as great as in some experiments of group 1, but the relatively upright position of the hydrochloric acid curve as compared with the relatively horizontal position of the sodium cyanide curve is maintained. In one experiment, although the shape of the curves is characteristic, their positions are reversed, so that the hydrochloric acid curve is to the left of the sodium cyanide curve.

Group 3. HCl, NaHCO3, NaCN. Vagi sectioned. Here again the general character of the curves shown in figure 1 is repeated. The irregularity of the respiratory tracings seen in sodium cyanide injections is eliminated with the section of the vagi and the tracings come to resemble those produced by hydrochloric acid injections.

Group 4. Lactic Acid, NaHCO3, HCl. Vagi intact. Figure 2 shows the effects of injection of lactic acid and hydrochloric acid on oxidations and ventilations. Two injections of lactic acid were made. In the first, 200 cc. were injected over a period of eight minutes and forty-seven seconds followed by 75 cc. of sodium bicarbonate. In the second, 180 cc. were injected during ten minutes and seventeen seconds, and followed by 66 cc. of sodium bicarbonate. Rapid injections (1.27 cc. as compared with 0.45 cc. per kilogram per minute 0.50 M hydrochloric acid injection) were necessary in order to obtain a stimulation in ventilation. Where the injections were not rapid, despite the large quantities injected, (400 cc.-500 cc.) stimulation in ventilation did not result, and the animal was left in a poor condition for further experimentation. Following the second sodium bicarbonate injection, a period of rest was allowed and 160 cc. of hydrochloric acid were injected.

Determinations *1*, *2*, and *3* show that the preinjection ventilation and oxidation remain approximately constant. Lactic acid is injected at the end of *3*. Oxygen consumption falls to 247 cc. from 335 cc. Ventilation increases from 9,314 cc. to 15, 485 cc. Oxidations begin to rise at the end of *4* reaching 302 cc. at the end of *6*, while ventilation increases to 38,992 cc. The break in the line between *6* and *7* again indicates an omission in determination of oxygen consumption because of an irregularity in the gradient. Sodium bicarbonate is injected during this omission. At *7*, oxidation increases to 396 cc. and ventilation falls to 14,391 cc. Ventilation falls farther at *8* to 11,777 cc. while there is a slight reduction in oxygen consumption. At the end of *8*, a second injection of lactic acid is made. There again is a marked reduction in oxidation to 269 cc. with ventilation rising to 15,688 cc. Ventilation then goes up to 21,280 cc. while there is little change in oxygen consumption at *10*. At *11*, oxidation increases to 310 cc. and is accompanied by an increase in ventilation to 33,510 cc. Injection is stopped at the end of *11*. At *12*, ventilation falls to 23,653 cc. and oxygen consumption increases to 325 cc. Sodium bicarbonate is injected during *13*. There is a slight reduction in oxidation accompanied

by a big drop in ventilation to 14,574 cc. At *14*, *15*, *16*, and *17* the oxidations and ventilations again assume approximately constant values. Hydrochloric acid is injected at the end of *17*. There is a small initial reduction in oxidation with an increase in ventilation from 13,577 cc. to 17, 418 cc. At *19* oxidation and ventilation increase followed by a diminution in oxidations and an increase in ventilation so that at *22* oxygen consumption is 303 cc. and ventilation is 24,887 cc. At *21* a deterioration of the animal's condition began which may account for the failure to attain a higher stimulation in ventilation. In the remaining experiments of this group, the effects of lactic acid agree in general with those just described. The main effect is a large increase in ventilation with relatively little or no impairment in oxidations. The large initial depression in oxygen consumption seen in figure 2 was not observed elsewhere.

In order to obtain an average relation existing between hyperventilation and impaired oxidation resulting from the hydrochloric acid and sodium cyanide injections, a series of quotients was computed by dividing the increase in ventilation in cubic centimeters per kilogram hour above the resting value by the cubic centimeters per kilogram hour of impairment of oxidations below the resting value. Obviously, in the three instances where the initial and final effect was an increase in oxidation, quotients could not be obtained. Twenty quotients for hydrochloric acid injections and twenty-five quotients for the sodium cyanide injections were obtained. The average value for the former was 198.9 and for the latter 76.9. These results indicate that the increase in ventilation per unit of impaired oxidation is 2.58 times greater in the case of hydrochloric acid than in the case of sodium cyanide.

SUMMARY AND CONCLUSIONS

Twenty-seven experiments were performed to determine quantitatively the relation existing between hyperventilation and impairment of oxidations as produced by intravenous injection of sodium cyanide, hydrochloric acid, and lactic acid.

In the experiments where the effects of lactic acid and hydrochloric acid were compared, a substantial agreement was found to be present. Both injections were usually characterized by a large increase in ventilation with corresponding little or no impairment of oxidations.

When the effects of hydrochloric acid and sodium cyanide were compared, a distinct difference in response was found.

The hydrochloric acid effects were characterized again by a large increase in ventilation with little impairment in oxidation. In three instances there was from the beginning to the end of injection a progressive increase in oxidation associated with marked hyperventilation. Initial increase in oxidations above the preinjection level followed by impairment with continually increasing ventilation was also noted.

Sodium bicarbonate administration following hydrochloric acid as a rule effected a restoration of ventilation to nearly the preinjection level, oxidations remaining appreciably impaired.

With sodium cyanide injection there was on the whole greater impairment of oxidation although an initial increase in oxidation was present in 83 per cent of the injections.

Both increasing and decreasing oxidations were associated with increasing pulmonary ventilation. During the phase of decreasing oxidations the increase in pulmonary ventilation per unit reduction of oxidations was relatively small.

Dividing increased ventilation by impairment of oxidations in all experiments in which oxidations were reduced gave a ventilation-impairment of oxidation quotient for hydrochloric acid of 198.9 and for sodium cyanide 76.9. These findings indicate that the increase in ventilation per unit of impairment of oxidations was approximately 2.58 times greater with hydrochloric acid injection than with sodium cyanide.

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AN ACCESSORY REGULATION OF ACID-BASE EQUILIBRIUM AND PULMONARY VENTILATION BY VIRTUE OF THE LACTIC ACID CHANGES IN THE LIVER

GEORGE S. McCLURE

From the Department of Physiology, University of Michigan, Ann Arbor, Michigan

Received for publication September 1, 1931

In previous papers from this laboratory it has been indicated that the hydrogen ion concentration of the neurones of the respiratory mechanism may be a significant factor in the control of pulmonary ventilation under various conditions. Accordingly any influence tending to modify the acid base equilibrium of the blood and tissues should be a contributing element in this control. In this light we wish to consider the liver as an accessory organ in the control of ventilation and tissue respiration. The absorption of lactic acid by the liver from the blood as demonstrated by Himwich, Koskoff and Nahum (1928) is the point involved.

When Hill and Meyerhof described the nature of the chemical processes involved in the lactic acid cycle occurring in muscle with lack of oxygen and subsequent recovery, the results seemed to indicate a more rapid total production of acid by the tissues during impaired oxidations than during normal oxidations and a liberation of base during recovery which was out of proportion to the formation of oxidative carbon dioxide (Gesell, 1923a, 1923b, 1923c). The possible significance of such changes in lactic acid metabolism on the acid base equilibrium and on the activity of the respiratory center was suggested. In a similar way, a gross participation by the liver in such a lactic acid cycle might be a significant factor in the general control of the acid base equilibrium of the body by exerting a constant effect upon blood and tissue pH, tissue oxidations and pulmonary ventilation. Should the liver take up lactic acid from the hepatic arterial blood and portal venous blood and consume it as a food there would be an acid base disturbance represented by the formation of three molecules of carbonic acid and the liberation of one free sodium ion. The total effect would be an increase in free and combined carbon dioxide and an increase in the hydrogen ion concentration of the emerging blood. On the other hand, should the lactic acid be converted to its precursor state without increased oxidation there would be a liberation of free base with a resultant increase in combined carbon dioxide, a decrease in free carbon dioxide and a reduction in the hydrogen ion concentration of the blood. By virtue of

such chemical processes the liver should exert a continual alkaline effect by opposing the accumulation of lactic acid just as the lungs exert a continuous alkaline effect by the elimination of carbon dioxide.

The probability of the liver being more important as a regulator of acid base equilibrium when oxidations are impaired and when lactic acid has accumulated is considered. This is put to a preliminary test by a study of the hydrogen ion concentration of the blood entering and leaving the liver before and after intravenous injection of sodium cyanide. The test assumes that reconversion of lactic acid into its precursor state is relatively unimpaired by cyanide poisoning.

METHOD. Dogs weighing from 6 to 10 kilograms were used, for the reason that they were more successfully operated upon than either smaller or larger dogs. The animals were anesthetized with morphine and urethane and a blood pressure cannula was placed in the carotid artery. A tracheal cannula was inserted and connected to a rebreathing tank through a soda lime cartridge for the removal of carbon dioxide. An injecting cannula was inserted into the femoral vein. An abdominal incision was made along the linea alba extending four and one-half inches downwards from the xiphoid process. In later experiments it was found that a transverse incision at the level of the tenth costal cartilage across the entire belly wall was more desirable. Though it caused slightly more hemorrhage, it gave access to the portal vein with the minimum amount of manipulation of the intestine. A metal guard consisting of a 5 inch length of brass tubing $\frac{5}{32}$ inch outside diameter and $\frac{1}{64}$ inch wall, grooved and leveled at one end, with holes through the tips for ligatures, was sutured to the portal vein and allowed to extend outside the abdominal wall. The incision was temporarily closed with hemostats and covered with saline soaked gauze. Samples of portal blood were taken through this guard by means of an 8 inch, number 22 syringe needle.

Samples were taken directly from the hepatic vein without the use of a guard. The wound was opened and the liver retracted forwards and downwards with the left hand so as to expose the hepatic vein. The sample was drawn from the vein as closely as possible to its point of exit from the liver with an 8 inch, number 22 needle. The syringe was held in the right hand and the needle was guided by the first and middle fingers of the left hand, the remainder of the hand holding the liver in place for sampling.

The arterial samples were taken directly from the femoral artery with regulation number 20 hypodermic needle. Excess hemorrhage was prevented by carefully inserting the needle into the arterial wall at an angle of about 15°.

The samples were transferred from 1 cc. tuberculin syringes to specially designed pH tubes (Gesell, 1928) and stored on ice. The samples were taken at 10 and 20 minute intervals after each injection of sodium cyanide

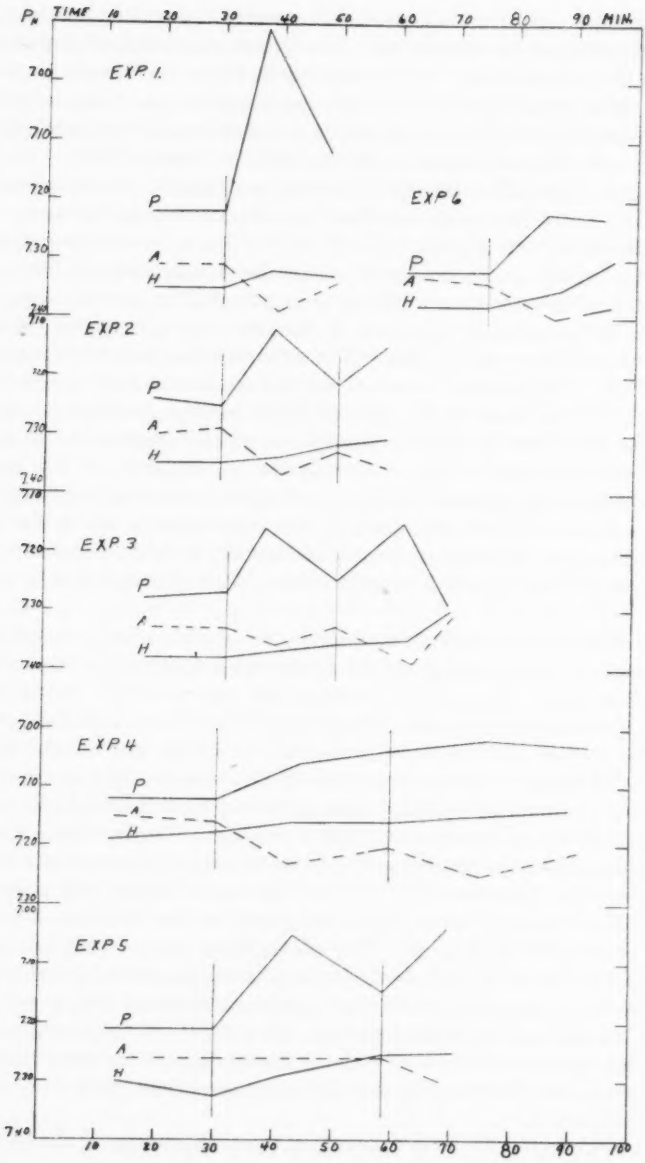
(0.001–0.003M. solution). Two sets of samples were taken before injection to be used as normal controls. The sodium cyanide was administered through the femoral vein. At the conclusion of the experiment the blood samples were centrifuged and the plasma removed into 1 cc. tuberculin syringes sealed with oil. The pH was then determined by the quinhydrone electrode according to the procedure described by Gesell (1928).

RESULTS. The pH of the portal, arterial and hepatic venous blood was plotted on the ordinates against time in minutes on the abscissae. The pH values have been plotted inversely so that an upward slope indicates increasing acidity, and a downward slope, decreasing acidity. The point of injection of the sodium cyanide has been indicated by a vertical line.

In the first experiment two sets of samples were taken before any injection of sodium cyanide. The pH of arterial blood was 7.31 and of the portal 7.22. The hepatic venous blood had the lowest hydrogen ion concentration of the three (pH 7.35) although having passed through the liver and intestines it might naturally have been expected to have the highest concentration. This greater degree of alkalinity of the hepatic venous blood, as compared with the portal blood, even under resting conditions may possibly be explained by the removal of some of the lactic acid by the liver, for blood lactic acid is relatively high under acute experimental conditions (Gesell, Krueger, Gorham, and Bernthal, 1930a, 1930b, 1930c).

After the injection of sodium cyanide the hydrogen ion concentration of the hepatic venous and portal blood increased while that of the arterial blood decreased. The greater hydrogen ion concentration of the portal blood was probably due to the diffusion of lactic acid into the blood stream from the tissues suffering from impaired oxidations due to the sodium cyanide. From the experiments of Gesell, Krueger, Gorham and Bernthal (1930b) it has been shown that there is an accumulation of lactic acid in the blood following injection of sodium cyanide. The hydrogen ion concentration of the hepatic venous blood also increased but to a lesser degree. Previous to the injection of cyanide the difference between the portal and hepatic blood was 0.13 pH. Eight minutes after the injection this difference had increased to 0.41 pH. The portal blood had at this time reached a pH of 6.91 which is near the limits at present considered as compatible with life, while the pH of the hepatic venous blood was 7.32, a value still within the limits of normal variation. The decreased hydrogen ion concentration of the arterial blood was presumably due to the removal of carbon dioxide from the blood by the excess ventilation resulting from sodium cyanide injection.

Eighteen minutes after the injection of the sodium cyanide, the hydrogen ion concentration of the portal blood was diminished but it was still 0.20 pH more acid than before the injection. The marked decrease in hydrogen


TIME
10
20
30
40
50
60
70
80
90 MIN.
EXP 1.
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A
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EXP 6
EXP 2
EXP 3
EXP 4
EXP 5

Fig. 1

ion concentration from pH 6.91 to 7.12 may be interpreted as a recovery phenomenon as is indicated by a stabilized blood pressure near the original level, and the return of regular moderate ventilation. It is also known that lactic acid disappears from the blood stream during recovery following cyanide injection (Gesell, Krueger, Gorham and Bernthal, 1930b). The hepatic blood after its primary increase in hydrogen ion concentration remained at a fairly constant level (fig. 1). The hydrogen ion concentration of the arterial blood increased though not reaching its former level. This may be interpreted as due to both the subsiding ventilation, and accumulation of fixed acid as might be expected in impaired oxidations, where the increase of lactic acid in the blood surpasses the effects due to an excess elimination of carbon dioxide.

In experiment 2, as is true for all experiments, portal samples showed a higher hydrogen ion concentration than the corresponding arterial and hepatic samples. In this experiment the initial difference in the three samples was not as marked as in the first experiment, nor were the differences that developed after the injection of sodium cyanide, as great as in the first. Neverthelss there is a noteworthy fall in the pH of the portal blood, with only a slight fall in the hepatic blood. As in the previous experiment, there was a tendency for the portal blood to return towards its preadministration pH level. The pH of the hepatic blood however continued to fall.

In the third experiment the reactions were very similar to those of the second, except that the pH of the arterial blood decreased. This was to be expected as the hyperpnea following the first injection of sodium cyanide was not marked. A second injection however did result in a marked hyperpnea, with a corresponding greater pH of the arterial blood.

The next experiment differed from the three preceding ones in that the pH of the portal blood did not return towards its preadministration level. The hepatic blood also showed a general tendency to increased hydrogen ion concentration, though the rise was much slower than that of the portal blood.

In experiment 5 while the usual rise in hydrogen ion concentration of portal and hepatic blood was obtained, the pH of the arterial blood remained constant until the second injection of cyanide. The second injection of sodium cyanide resulted in the usual hyperpnea hence the increased pH of the arterial blood.

In the following experiment (no. 6), the changes, though less abrupt, were the same in general as were found in experiments 2, 3 and 4. The maximum hydrogen ion concentration of the portal blood in this experiment was pH 7.22, while the corresponding hepatic sample had a pH of 7.35. The difference of 0.13 pH though not as marked as in the first experiment is real and significant being at least 0.1 pH beyond the limits

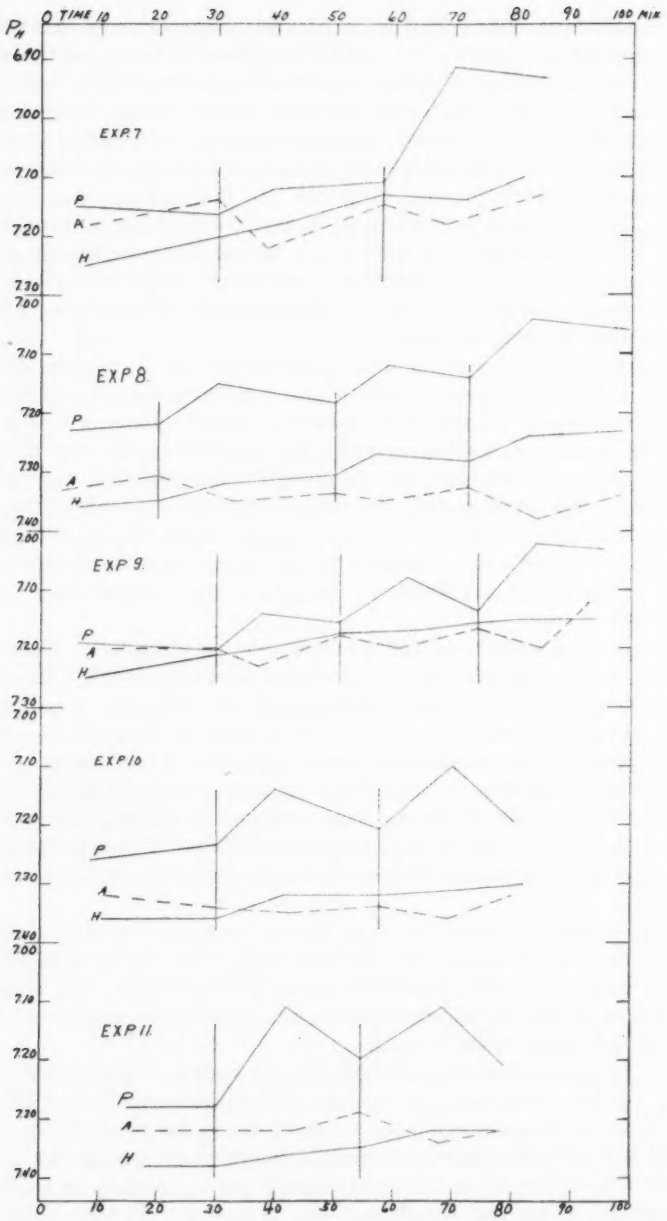

Fig. 2

of error of method. In this experiment while the portal blood showed a tendency to recovery the increase in hydrogen ion concentration of the hepatic blood continued to a greater extent than usual. Possibly the function of the liver has been affected.

In all previous experiments the relationship between portal arterial and hepatic venous bloods during normal basal condition has been uniform. That is, the portal blood had the highest hydrogen ion concentration, the hepatic blood the lowest and the arterial blood had a value between the two.

In experiment 7 the usual order obtains at the start, but in the second series of samples the hydrogen ion concentration of the arterial blood was the highest. As this is the only case in which the hydrogen ion concentration of the arterial blood becomes higher than that of portal blood, the determinations may be in error. However, the animal was not in stable equilibrium as is indicated by the initial decrease in pH of the hepatic blood and increase in pH of the portal blood. With the injection of cyanide the pH of the portal blood decreased slightly and that of the hepatic blood continued to decrease at about the pre-injection rate. Due to the marked hyperpnea the pH of the arterial blood increased. After the second injection of cyanide, a marked decrease in pH of the portal blood was observed, accompanied by an insignificant change in the hepatic blood. As usual the hydrogen ion concentration of the arterial blood increased.

In experiments 8 and 9, three injections of sodium cyanide were administered in each. After each injection the hydrogen ion concentration of the arterial blood increased due to the excessive ventilation, while that of the portal and hepatic venous bloods decreased, the hepatic more slowly than the portal. The effects of each injection were increased pH of the arterial blood and a marked decrease in the portal blood, while the pH of the hepatic blood changed more gradually. The effects of the successive injections of cyanide were superimposed upon one another. The hydrogen ion concentration of the portal blood rose higher and higher. The hepatic blood showed similar changes yet lagged behind the increase of the portal blood.

In experiment 8 due to excessive ventilation the total effect on the arterial blood was a decrease in hydrogen ion concentration while in experiment 9 although each injection was accompanied by a decreased hydrogen ion concentration of the arterial blood, the late rise of the arterial blood in each case was so marked that the hydrogen ion concentration of the arterial blood reached a level above that preceding the injections.

In experiment 10 similar results were obtained. Initial differences in pH between portal and hepatic venous blood increased with each injection but the final differences after each injection were not much affected.

In experiment 11, the effects of cyanide on the portal and hepatic blood were the same as usual.

Discussion. The fact that removal of the liver leads to an increase in the blood lactic acid and a reduction in the carbon dioxide capacity of the blood (Kilborn, Soskin and Thomas, 1928), that feeding of sodium lactate increases the glycogen content of the liver (Cori and Cori, 1929), and that the liver extracts lactic acid from its blood supply (Himwich, Koskoff and Nahum, 1928) support the conclusion that the permanent basal differences and the greater temporary difference in hydrogen ion concentration between the portal and hepatic blood following cyanide injection are due to reconversion of lactic acid into glycogen by the liver with a liberation of free base.

According to the data of Himwich, Koskoff and Nahum (1928) the average difference between portal and hepatic blood in these experiments was 14 mgm. for 100 cc. If it is assumed that the lactic acid removed was in the form of sodium lactate, the base equivalent would be 14/4 or 3.5 volume per cent of carbon dioxide. The Hasselbalch equation yields the following:

$$pH = P_k + \text{Log} \frac{NaHCO_3}{H_2CO_3}$$

If the pH of hepatic venous blood is 7.32 and $NaHCO_3$ is equivalent to 40 volumes per cent of carbon dioxide an average for venous blood, then:

$$7.32 = 6.1 + \text{Log} \frac{40}{H_2CO_3}$$

$$H_2CO_3 = 2.4 \text{ volumes per cent.}$$

If base equivalent to 3.5 volumes per cent has been added to the portal blood as it passes through liver then the sodium bicarbonate of the portal blood will have been 36.5.

Then:

$$pH = 6.1 + \text{Log} \frac{36.5}{2.4}$$

$$pH = 7.28$$

That is, portal blood at a pH of 7.28 loosing 14 mgm. of lactic acid per 100 cc. attains a pH of 7.32. This represents a decrease in hydrogen ion concentration of 0.04 pH. A reconversion of 28 mgm. of lactic acid which would produce a decrease in hydrogen ion concentration of 0.09 pH would agree more closely with the average differences in pH for basal conditions obtaining in our experiments. The effect of the liver on pH of the total mixed circulating blood should of course be smaller and vary roughly with the ratio of liver volume flow to cardiac output. Liver volume flows of

20, 30 or 40 per cent of the total flow of blood accompanied by lactic acid reconversion of 28 mgm. of lactic acid per 100 cc. of blood should be associated with a decrease in hydrogen ion concentration of the blood as a whole with each complete circulation of the blood of the following approximate values, 0.018, 0.027, and 0.036 pH. Computing on this basis, the larger local hepatic changes in pH accompany cyanide injection, whatever the chemical cause may be, must produce proportionately larger changes in the hydrogen ion concentration of the whole mixed blood. It should be added however that no experiments were performed to determine the proportion of portal flow to cardiac output or whether the portal flow increased or decreased under the conditions of our experiments with the administration of sodium cyanide. Quantitative evaluation of the significance of the liver in relation to the acid base equilibrium of the body must, therefore, await further experiments.

SUMMARY

An attempt has been made to study the function of the liver in the control of the acid-base equilibrium.

The assumption that absorption and reconversion of lactic acid by the liver to glycogen or sugar is accompanied by a liberation of free base and a reduction in hydrogen ion concentration was tested by pH determinations of portal, arterial and hepatic venous blood.

The assumption that the magnitude of this reconversion of blood lactic acid and liberation of free base would vary with the degree of impairment of oxidation and concentration of blood lactic acid was tested by similar pH determinations following intravenous injection of sodium cynide.

Under the basal conditions obtaining the hydrogen ion concentration was lowest in the hepatic venous blood, highest in the portal blood and midway between in the arterial blood.

Intravenous injection of sodium cyanide produced variable effects on the hydrogen ion concentration of the arterial blood depending on the accompanying changes in pulmonary ventilation. The greater the augmentation of ventilation the greater was the initial decrease in hydrogen ion concentration.

Intravenous injection of sodium cyanide produced a large increase in the hydrogen ion concentration of the portal blood and a relatively small increase in the hepatic venous blood. The resulting increase in the difference of hydrogen ion concentration between portal and hepatic venous blood occurred with and without an accompanying decrease in hydrogen ion concentration of the arterial blood.

The fact that hepatic venous blood showed a lower hydrogen ion concentration than the portal or arterial blood during "normal basal conditions" suggested that the liver is continually removing lactic acid from the blood and liberating free base.

The fact that the difference in hydrogen ion concentration between portal and hepatic venous blood increased after intravenous injection suggested that this process is augmented during impaired oxidation.

The liver thus provides a self corrective mechanism against acidosis arising from impaired oxidation.

The resupply of sugar to the blood and tissues resulting from the reconversion of lactic acid is considered as an accessory mechanism supporting the failing oxidations.

Assuming a reconversion of 28 mgm. of lactic per 100 cc. of blood, enough base is liberated to lower the hydrogen ion concentration of the portal blood about 0.09 pH. This approximates the difference in pH of portal and hepatic venous blood of the "normal basal condition" found in these experiments.

Assuming that the flow of blood through the liver equals 20, 30 or 40 per cent of the total cardiac output it is computed that the continuous effect of the liver on the hydrogen ion concentration of the total mixed blood should be approximately a reduction of 0.018, 0.027 or 0.036 pH with each complete circulation of the blood.

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LATE EFFECTS OF SECTION OF THE VAGUS NERVES ON GASTRIC ACIDITY

FRANCES R. VANZANT[1]

From The Mayo Foundation, Rochester, Minnesota

Received for publication September 17, 1931

Although numerous observations have been made on the effect on the stomach of section of the vagus nerves, they have been made for only short periods after operation. Pieri, Lapenna, and Tanferna cut both vagus nerves either above or below the diaphragm in eight cases, and continued to study motility and secretion of the stomach for as along as two years afterward. They found that the decreased motor activity which was present at the beginning gradually disappeared after three or four weeks. Similarly the acidity which at first was lowered returned to normal after a few months.

Hartzell (1929) reported the effect of vagotomy on gastric secretion of the dog. He used a test meal consisting of 80 grams of finely ground horse meat in 250 cc. of water. Specimens were removed with a tube every fifteen or thirty minutes until the stomach was empty. This work was repeated frequently until the dogs were thoroughly accustomed to the tube and no longer showed any emotional reaction to the procedure. After this preparatory work had been done and the individual peculiarities of gastric secretion were known for each dog, both vagus nerves were cut in the thorax. Following this Hartzell found marked reduction in both free and total acidity which persisted through a five-months period of observation. At times free acid did not appear at any stage of digestion. In every animal the highest curve of acidity obtained post-operatively was lower than the lowest curve obtained pre-operatively. In each case the stomach began to empty less promptly than normally, but the total emptying time was only slightly prolonged. In two animals in which the vagus nerves were sectioned in the abdomen, there was no constant reduction in acidity, but there was some acceleration of emptying time.

This paper is based on observations made on four of the eight dogs used by Hartzell in the studies mentioned. All of these animals were operated on during February, 1929. My observations were made between January and May, 1931, about two and a half years after the vagotomy. The vagus nerves of three of the dogs had been sectioned in the thorax.

[1] Fellow in Medicine, The Josiah Macy, Jr., Foundation.

The nerve fibers of the fourth dog had been cut along the lesser curvature of the stomach and the pyloric branch of the hepatic nerve had been sectioned. In addition an incision had been made through the gastric wall down to the mucosa, encircling the stomach a short distance below the cardia. In suturing this incision the edges were inverted, so as to bring the serous surfaces together and thus to prevent the severed nerve fibers from reuniting.

In the interval between Hartzell's work and the beginning of my observations the dogs were kept in kennels on a diet of dog biscuits and a mash made of meat and cereal. Of the three dogs with the vagus nerves cut in the thorax, one (dog 1) remained in excellent physical condition and appeared normal in every way except for a sclerodermatous change about the skin of the mouth. The other two dogs became thin and anemic, and decubitus developed. It is doubtful if these changes could be ascribed to vagotomy, for when these animals were given fresh meat and milk in addition to the usual kennel ration, they gained weight and improved generally. Following a period of active treatment of the skin, the ulcerations of dog 2 healed; the condition of dog 3 never entirely cleared up, and it finally became worse in spite of treatment. All three animals had excellent appetites. Dog 2 was subject to chronic diarrhea; the stools were soft and unformed and contained a large amount of mucus.

Method of experiments. Fractional gastric analyses were made according to Hartzell's technic. The secretion was also studied following subcutaneous injection of 1 mgm. of histamine. Roentgen-ray examinations were made to determine the size of the stomach and rate of emptying after the ingestion of a meal consisting of a suspension of barium in acacia and milk.

Results. In dog 1 the value for both free and total acid were found to be uniformly higher than they were immediately after operation. At times they were practically the same as before operation but the average curve was slightly lower than the average pre-operative curve (fig. 1). In dogs 2 and 3, free acid did not appear during the time when they were in poor condition. As they became more healthy both total and free acidity increased until the curves were practically as high as those seen before operation. The digestive period was somewhat shorter than before the nerves were sectioned.

With histamine the gastric acidity and volume of secretion were about a third lower than they usually are in normal dogs, but the response of these particular experimental animals to histamine was not determined before vagotomy. Although measurement could not be made of the amount of mucus secreted, it was definitely more abundant than in normal animals. The giving of 0.8 mgm. of atropine sulphate hypodermically half an hour before the injection of histamine reduced both the acidity and the volume

of secretion by 25 per cent. This is comparable with the reduction seen in normal animals. The injection of 6 mgm. of pilocarpine subcutaneously did not seem to have any influence on gastric secretion.

Dog 4, in which the branches of the vagus nerves had been cut below the diaphragm, was in excellent condition and the curve of acidity was still practically normal, as it was shortly after the operation (fig. 2). Strange

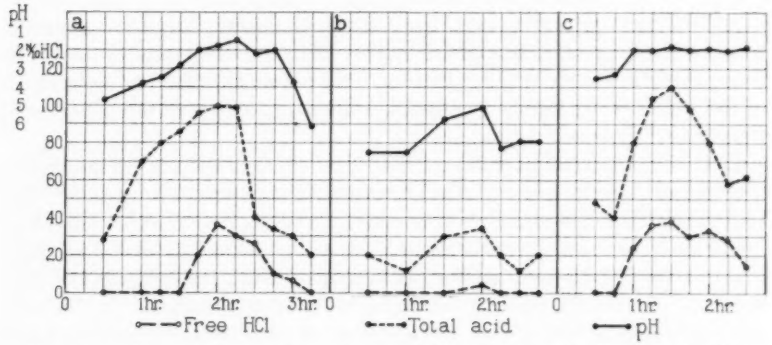

Fig. 1 (dog 1). The curves of acidity: *a*, before operation, *b*, shortly after intrathoracic vagotomy, and *c*, two years later.

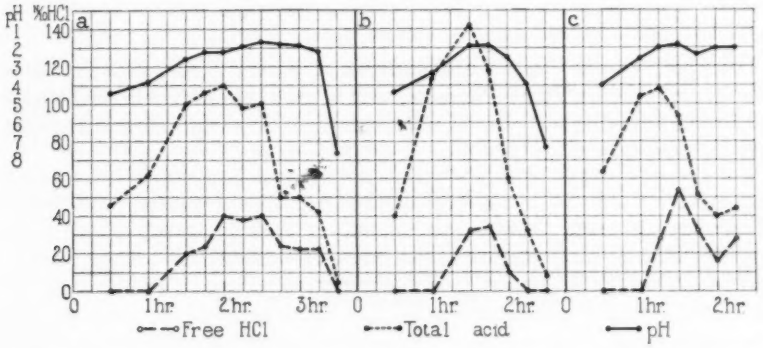

Fig. 2 (dog 4). The curves of acidity: *a*, before operation, *b*, shortly after subdiaphragmatic vagotomy, and *c*, two years later.

to say the response to histamine was subnormal, just as it was in the other three animals. The responses to atropine and to pilocarpine were those of a normal dog.

With the barium meal, motility seemed normal except for the fact that in dog 4 the stomach, which seemed at first to be emptying at the usual rate retained a small residue for several hours. This peculiarity of emptying has been described by Hughson.

In order to determine if the severed ends of the nerves had reunited, dogs 1 and 2 were operated on again. Regeneration of the nerves had not taken place. Dog 3 was killed, and at necropsy a firm fibrous band was found to connect the ends of one nerve, but on histologic examination the nerve was found to be entirely degenerated and anastomosis had not taken place between the cut ends.

COMMENT. Hartzell studied the gastric acidity in several dogs for five months after vagotomy to see if the marked diminution in gastric secretion which at first appeared was due to loss of autonomic influences or merely to the immediate effects of the operation. He concluded that the destruction of the vagus nerve supply resulted in marked reduction in the capacity of the stomach to secrete acid.

Pieri and Tanferna have found that such changes are not permanent in the human subject, and my study shows that the same is true in the dog. It appears that the stomach is so nearly autonomous that its secretory functions will return almost to normal some time after bilateral vagotomy. It is impossible to determine the mechanism of readjustment from the data at hand, but this question is now being investigated further. In this connection it may be noted that the injection of pilocarpine did not produce the same increase in gastric secretion as is seen in normal dogs, whereas atropine did produce the usual decrease in secretion.

SUMMARY

In dogs there is a marked dimunition in gastric secretion which lasts for several months after bilateral section of the vagus nerves in the thorax. Later the secretion increases so that after two or three years it becomes approximately normal.

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THE INFLUENCE OF DIET AND THE ANTERIOR PITUITARY GROWTH HORMONE ON THE GROWTH RATE OF ADOLESCENT RATS

A. HUGHES BRYAN AND DAVID W. GAISER

From The Laboratory for Surgical Research, Harvard Medical School

Received for publication August 15, 1931

The acceleration and retardation of the growth rate of albino rats has been attacked largely from the two points of view of nutritional and endocrinological research. Many workers have studied the influence of such dietary factors as amino acids, vitamines and mineral elements on the growth and general nutrition of laboratory rats, and it is well known that these important substances must all be present in appropriate quantities if the growth of the young rat is to be insured. Acceleration of the growth rate has been produced by Evans (5) and his co-workers in the case of the rat and by Putnam, Benedict and Teel (1) in the case of the dog by the injection of anterior pituitary growth hormone. Smith (2) has successfully ablated the pituitary in rats and has demonstrated that implantation of anterior pituitary substance offers an entirely successful replacement therapy. He has also found that injection of the growth hormone of Evans will cause the hypophysectomized animal to return to a normal growth rate. In this investigation we have sought to combine these two methods of stimulating growth, namely, the use of a complete special diet and the injection of anterior pituitary growth hormone, in an attempt to approach the maximum rate of growth in the rat.

Osborne and Mendel (3) studied the rate of growth of male albino rats as influenced by various diets, using as data the records of their colony for the previous fourteen years, as well as results obtained with a group of special diets. Donaldson's (4) standards indicate that the male albino rat grows from 60 to 200 grams in 79 days. A study of Osborne and Mendel's diagrams (similar to fig. 2 of this paper) of the performance of selected groups of 25 of their stock male rats fed for the most part on a special dog biscuit with occasional offerings of fresh food, indicates, as they point out, that their stock rats showed from year to year a progressively more rapid and uniform growth. In 1912 the 25 selected males averaged 94 days to gain from 60 to 200 grams with the curves fanning out from 50 to 150 days. In 1919 the average was 70 days with extremes of 30 and 120 days and in 1925, 25 animals averaged 67 days with extremes of 30 and 110 days.

Osborne and Mendel consider that this improvement may be due either to the influence of the rapid advances made in nutritional science during those years, or to the effect of breeding. But the ability of special diets to accelerate the growth rate was most marked. In 1922 such a diet was constructed, consisting of red dog flour, meat scrap, casein, alfalfa and sodium chloride and designed to contain protein of good biological quality, vitamines and mineral elements in properly balanced amounts. When fed to young male rats this diet caused a more uniform and rapid growth than had been observed in the stock rats fed with dog biscuit. The 25 rats selected from those receiving this diet required an average of only 38 days to grow from 60 to 200 grams with extremes of approximately 25 and 49 days. Further experiments showed that rats grow somewhat better on a diet containing 21 per cent or more protein and on the basis of these considerations special diets were constructed which caused even more uniform and rapid growth than the one containing red dog flour. Diet B of this paper is one of the latter diets. Osborne and Mendel conclude from these studies that when fed a proper ration, the rat is capable of a much improved rate of growth over that ordinarily obtained in the laboratory.

Evans (5) in a well known investigation prepared an alkaline extract of the anterior lobe of beef pituitaries and injected it intraperitoneally into young female rats using their litter mate sisters as controls. Almost from the beginning the animals receiving the anterior hypophyseal fluid outgrew their controls but their superior gains were most marked in the post pubertal period when they were from 90 to 150 days old. At this time the growth of the injected rats did not undergo the sudden decline characteristic of that of normal animals, but continued on, in some cases almost unabated. Mendel and Cannon (6) showed from Evans' protocols that 20 of Evans' injected females grew from about 60 to around 200 grams in 30 to 40 days with an average of 35 days. Thus their average daily gain was 4.9 to 3.7 grams. Using the improved diets published in the previous paper (3), Mendel and Cannon found that their 20 females which grew the most rapidly averaged 4.0 to 2.6 grams per day, and grew from 60 to 200 grams in 35 to 54 days, figures which compare favorably with those obtained by Evans. On the basis of this comparison, together with the fact that Evans emphasized the more pronounced influence of pituitary growth hormone in the later or post-pubertal period, Mendel and Cannon suggest that the pituitary growth hormone may "serve primarily to modify adult development, rather than the growth of the more typically adolescent period".

The present investigation was undertaken at the request of Dr. Harvey Cushing, in an attempt to determine whether or not the pituitary growth hormone could further accelerate the already rapid growth of young rats fed one of Mendel's special diets. Doctor Mendel had originally raised this question in a personal communication to Doctor Cushing.

EXPERIMENTAL METHODS. The young male rats used in this experiment were weaned at 30 days and placed in individual cages a few days before they attained the weight of 60 grams. Our rats are a cross-breed of pied and albino stock and the males usually reach the weight of 60 grams on the 29th to 36th day of life, which is somewhat earlier than Donaldson's standard of 50 days. Two diets were used in this investigation. Diet B is one of Mendel's special diets published in 1926 (3) and the other is a synthetic ration also devised by Mendel (7) which we chose partially because we felt it would cause a less rapid growth and hence control the results obtained with diet B. The composition of the two diets follows:

Diet B

	per cent
Yellow corn meal	58
Skim milk powder	29
Casein	12
Sodium chloride, Ferric citrate, Potassium iodide	1*

	grams
*NaCl	93.0
Fe citrate	6.34
KI	0.02

This mixture as a dry powder was given to the rats *ad liberatum*. In addition each rat received daily, in a separate dish, a supplementary feeding of approximately 0.8 gram of dried ground beef liver. For the most part the liver was completely consumed, but not infrequently it would go untouched for several days without any apparent deleterious effect on the rate of growth.

Diet C

	per cent
Entire wheat flour	65.8
Whole milk powder	32.8
Calcium carbonate	0.7
Sodium chloride	0.7

A supplementary feeding of a large lettuce leaf was given to each rat four to six times a week. The lettuce was always eagerly and completely eaten.

The growth hormone used in this experiment consists of several lots prepared for us by Parke, Davis & Company, following essentially the method published by Putnam, Teel and Benedict (8) with the further refinement of Teel's process of salting out with 20 per cent sodium sulfate (9). A detailed account of the preparation may be found in a recent paper by Bugbee and his co-workers (11).

As far as possible littermate brothers were used in the four parts of the experiment. Two groups of young rats were put on diet B, one of these groups was treated with hypophyseal fluid and the other served as a control

group. The same procedure was carried out with diet C. The injected animals of both diet groups first received the hormone in doses of one-half cubic centimeter daily, increasing to one cubic centimeter daily. It was soon found that a somewhat larger dose, injected twice daily, gave more consistent results. Thus the injected animals during all but the very first part of the experiment received, on reaching 60 grams, one-half cubic centimeter twice daily intraperitoneally, this was increased to one cubic centi-

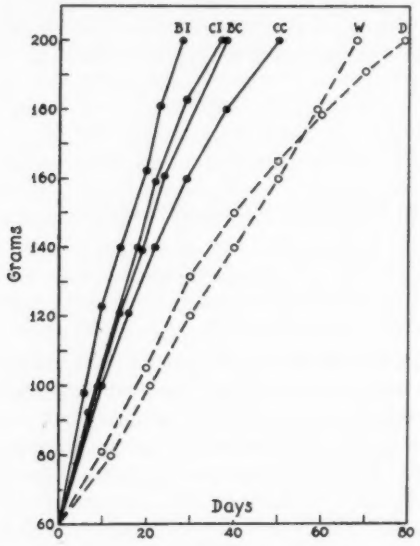

Fig. 1. Growth curves for the four rats which grew from 60 to 200 grams in the average number of days required by their respective groups. *BI*, average rat of the diet B injected group. *BC*, average rat of the diet B control group. *CI*, average rat of the diet C injected group. *CC*, average rat of the diet C control group. *W*, curve for the Wistar Colony published by Greenman and Duhring (10). *D*, Donaldson standard curve (4).

meter twice daily at 100 grams, one and one-half cubic centimeter twice daily at 150 grams and two cubic centimeters twice daily at 180 grams.

All the rats in all four groups were weighed daily.

Results. The number of days required by male rats to grow from 60 to 200 grams has been determined in the case of 34 animals fed on diet B and treated with growth hormone, 33 animals fed with diet B but not injected, 27 animals fed with diet C and injected and 22 diet C controls. The average number of days required by the four groups to complete the span was: diet B injected, 28 days; diet B controls, 38 days; diet C injected, 37 days: and diet C controls, 50 days. Figure 1 shows these average curves com-

pared with the Donaldson curve (4) and the curve for the Wistar Colony as published by Greenman and Duhring (10).

But these averages are only of limited significance and the figures which follow show in a more convincing way the superior growth of the injected

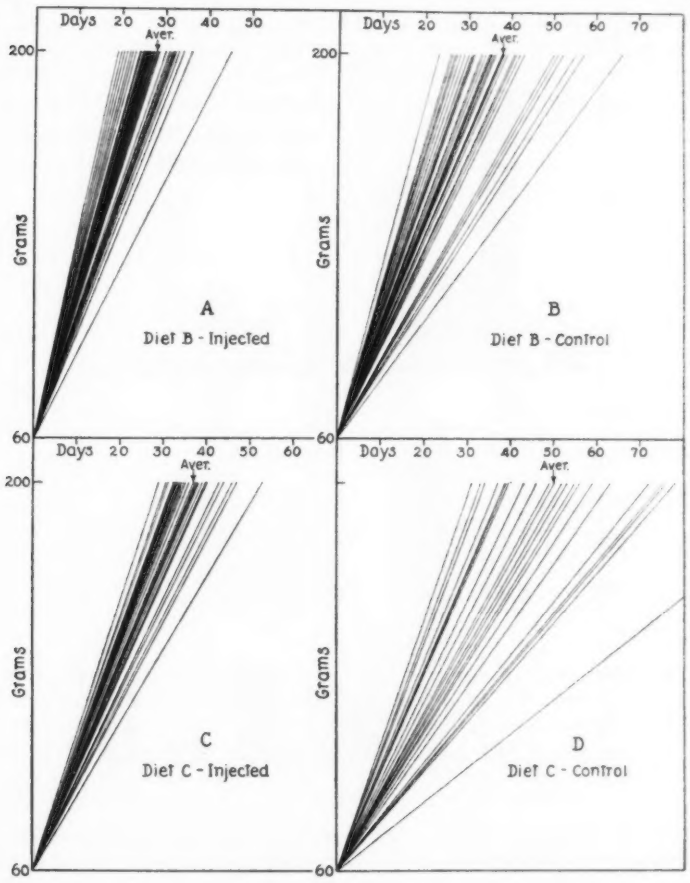

Fig. 2. Graph showing the number of days required by the rats in each of the four parts of the experiment to grow from 60 to 200 grams.

rats. In figure 2 are plotted, in the manner used by Osborne and Mendel, the number of days each rat required to grow from 60 to 200 grams. It will be noticed at a glance that the curves for the control rats in both diets B and C tend to fan out over a considerable number of days; those of the

better diet, however, are somewhat more concentrated in the range of 30 to 40 days. When we come to consider the curves for the injected rats fed with the two diets, the influence of the injection makes itself at once evident in the concentration of a majority of the curves in the portion of the graph

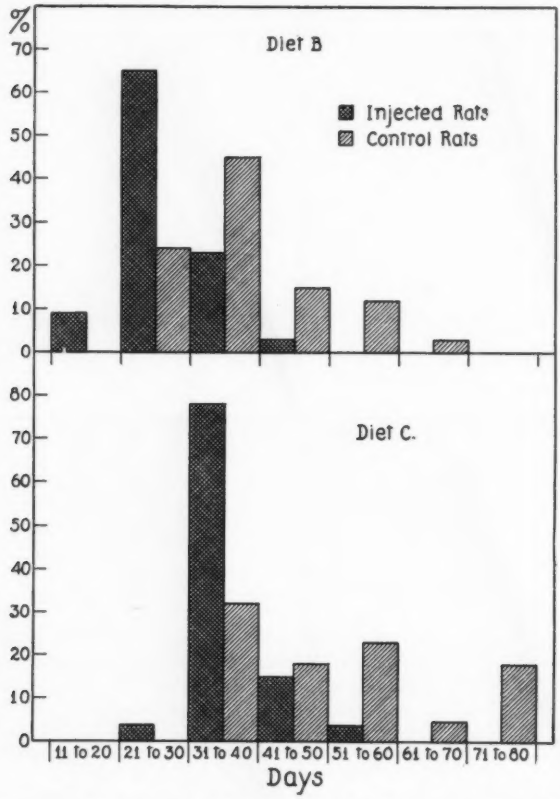

Fig. 3. Per cent of rats in each of the four parts of the experiment which attained 200 grams in periods of ten days each. Each group is calculated on the basis of 100 per cent. Thus 23 per cent of all the diet B injected animals, 45 per cent of all the diet B controls, 78 per cent of all the diet C injected animals, and 32 per cent of all the diet C controls required from 31 to 40 days to grow from 60 to 200 grams.

that precedes 30 days in the case of diet B, and 40 days in the case of diet C. Figure 3, which represents the per cent of rats in the four parts of the experiment which reached 200 grams within ten day periods brings out in a slightly different manner the superior growth shown by the injected rats.

In the first place the control rats on both diets tend to less uniform results than are obtained from those injected, as is shown by the fact that fewer controls attained a weight of 200 grams in any single period of ten days than was the case with the treated rats. In the second place a definitely larger number of the injected rats reached 200 grams in the lower periods of ten days, that is, before 30 days in the case of diet B and before 40 days in the case of diet C than was true of the controls. This is further shown by figure 4 where it will be noticed that 74 per cent of the diet B injected rats grew from 60 to 200 grams in less than 30 days while only 24 per cent of their controls did so; and there were 26 per cent of the injected rats reach-

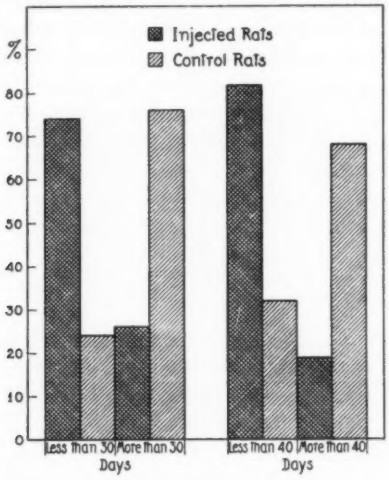

Fig. 4. The figure on the left shows the per cent of rats of the diet B injected and control groups which required less or more than 30 days to grow from 60 to 200 grams. The figure on the right gives similar information for the diet C animals except that 40 days is taken as the dividing line.

ing 200 grams after 30 days, and 76 per cent of their controls. In a similar manner 81.7 per cent of the injected rats fed diet C, and only 32 per cent of their controls grew to 200 grams in less than 40 days whereas it required more than 40 days for 18.7 per cent of the injected and 68 per cent of the controls to attain 200 grams. Finally, it is to be noticed from figure 3 that while the injected rats of both diet groups exhibited a uniformly better growth than their respective controls, the injected rats fed on diet B grew more rapidly than the injected rats receiving the poorer diet as is shown by the fact that 74 per cent of the former grew the necessary 140 grams in less than 30 days while 78 per cent of the latter group reached 200 in 31 to 40 days.

In the proper estimation of growth the body size of the animal should be taken into consideration as well as the gain in weight and the actual weight attained. It seemed to be within the realms of possibility that adiposity, edema, or perhaps a disproportionate growth of some sort might occur in the injected animals and that, therefore, their gain in weight might not be accompanied by an appropriate increase in size. We considered that the most important single measurement which we could make to rule out the possibility that some of our rats were heavier than they should be for their size would be the determination of the body length attained at 200 grams. Accordingly numerous representatives of each of the four groups, including both rapid and slow growers within the group, were killed upon reaching 200 grams, gently stretched out upon a table, and the distance from the tip of the nose to the anus was measured. The results of these measurements are shown in table 1. It will be noticed that regardless of which diet the rat was fed, whether or not he was injected, and how long it took him to gain the necessary 140 grams, his body length at 200 grams was 195 mm. within a

TABLE 1

	NUMBER OF RATS MEASURED	NUMBER OF DAYS REQUIRED FROM 60–200 GRAMS	AVERAGE BODY LENGTH	EXTREMES	
				High	Low
			mm.	*mm.*	*mm.*
Diet B Injected	18	19–33	195.4	203	187
Diet B Control	20	26–66	195.3	203	184
Diet C Injected	22	19–53	194.6	199	185
Diet C Control	7	31–72	195.6	201	186

reasonably small range of error. Donaldson's statistics indicate that at 200 grams a rat should have a body length of 202 mm., a figure slightly larger than ours. We are unable to explain why our rats should be very slightly shorter at 200 grams than the Donaldson standard.

Discussion. Three more or less independent factors seem to influence the growth rate of young rats. In the first place there is an unknown factor which causes some rats, no matter how fed or treated, provided of course, that their growth is not actually hindered, to outgrow their brothers, occasionally at a surprising rate. We are unable at this time to define this unknown stimulus to growth further than to say that some rats show a greater capacity for rapid growth than their brothers. Whereas 50 days were required on an average for the diet C controls to reach 200 grams, and the majority of this group needed more than 40 days, two rats of this group gained the necessary 140 grams in 31 and 33 days. This is rather striking because the second and third most rapid growers among the diet C injected animals required also 31 and 33 days to complete the span. In a similar

way the two best growers among the uninjected rats fed on diet B grew to 200 grams in 23 and 26 days whereas two-thirds of the diet B injected animals required 26 days or more for a similar gain. We did notice, however, that both the superior growth diet, and the administration of the anterior pituitary growth hormone, buffered to a certain extent the influence of this individual growth potential and thus caused a more uniform growth in all rats similarly treated. We feel that we may further suggest the possibility that a more potent preparation of the growth hormone might cause an even more uniform growth by a further reduction of the influence of this unknown growth potential.

The work of Mendel and his co-workers (3), (6) has proven adequately that the second factor which determines the growth rate of young rats is the quality of the diet, and the differences between our diet C and diet B controls corroborate Mendel's results. The animals fed on the better diet tend to both a more rapid and a more uniform growth rate.

Evans (5) observed that his injected females grew more rapidly than their control sisters almost from the beginning and our results obtained with the two groups of injected animals show that the growth extract causes a change in the growth curves similar to that observed in substituting a better for a poorer diet. The injected animals in both diets show a marked tendency to more uniform and rapid growth than is the case with their controls.

Most significant is the fact that the results obtained in this paper seem to indicate that these three factors are to a certain extent independent in their action. Thus, as stated above, certain isolated individuals outgrew their controls at a rapid rate regardless of the diet used, and they even tend to equal the best records of rats to whom extra pituitary growth hormone is supplied. The independent action of the diet is shown by the fact that the majority of diet B injected animals showed a more rapid growth than the majority of diet C injected animals. *The rapidity of the growth of animals subjected to an excess of hypophyseal growth hormone is, then, limited in a majority, if not all cases by the diet fed these animals, and also, to a lesser degree, by the individual growth potential of the rat under discussion.*

It might be supposed that in as much as the hypophysis is considered the chief endocrine regulator of growth, the process of keeping young rats at all times supplied with a maximum of the growth promoting principle of this gland should result in a uniform gain in weight if all other conditions could be kept constant. That this absolute uniformity of growth has not been realized in our injected rats is, we feel, due in all probability to the utter complexity of the subject. Not only must all the building stones of the body be supplied in the food in adequate quantities and proper proportions, but also the ability to rapidly digest, absorb and utilize this material must also be present. Young rats, however standardized, are probably

individuals, and when one considers the complexity of the growth function, it is not surprising that even in the presence of an excess of growth stimulator, increase in body weight does not occur uniformly in all animals under apparently constant external conditions.

The data at hand seem to indicate that the maximum average daily increase in weight of which a young male rat is capable over a considerable period of days lies in the neighborhood of six to seven grams. Mendel and Cannon (6) found that their 20 best growers among young males fed on their special diets averaged from 6.1 to 4.8 grams a day. They studied the very rapid gain in weight shown in their older experiments when a rat was allowed to return to normal conditions after having been stunted for a variable length of time by the absence of one or another growth essential. Consideration of 20 males so stunted at different weights showed that on resuming a normal diet they grew for a period of 7 to 14 days at a rate of from 7.6 to 5.9 grams a day. Table 2 presents the data from our best growers on the four parts of the experiment. It will be noticed that the diet B control animals grew from 6.1 to 4.0 grams per day, rates which are similar but not quite so rapid as those obtained by Mendel on the special diets. Our diet B injected animals, however, averaged from 7.4 to 5.4 grams a day, rates which are somewhat higher than those obtained by either Mendel or ourselves on special diets alone, and which are quite comparable to the 7.6 to 5.9 gram average daily performance which Mendel observed for shorter periods in his realimentation experiments. Consider-

TABLE 2

DIET B INJECTED			DIET B CONTROLS			DIET C INJECTED			DIET C CONTROLS		
Rat number	60-200 grams	Daily average	Rat number	60-200 grams	Daily average	Rat number	60-200 grams	Daily average	Rat number	60-200 grams	Daily average
	days			*days*			*days*			*days*	
140	19	7.4	52	23	6.1	203	29	4.8	71	31	4.5
132	20	7.0	130	26	5.4	101	31	4.5	103	33	4.2
136	20	7.0	126	26	5.4	195	31	4.5	55	34	4.1
104	21	6.7	102	27	5.2	187	33	4.2	83	37	3.8
168	22	6.4	94	27	5.2	85	33	4.2	135	39	3.6
144	22	6.4	143	28	5.0	81	33	4.2	95	39	3.6
116	23	6.1	44	29	4.8	73	33	4.2	91	39	3.6
182	24	5.8	212	30	4.7	61	33	4.2	123	43	3.3
216	25	5.6	142	31	4.5	57	34	4.1	21	46	3.0
50	25	5.6	16	31	4.5	69	34	4.1	63	49	2.9
92	25	5.6	76	31	4.5	163	34	4.1	79	50	2.8
100	25	5.6	68	33	4.2	191	35	4.0	149	52	2.7
184	26	5.4	110	33	4.2	181	35	4.0	115	53	2.5
96	26	5.4	88	34	4.1						
86	26	5.4	118	35	4.0						

ation of these facts leads us to believe that the more rapid growers among the diet B-injected animals are approaching, at least, the upper limit of rapidity of growth in adolescent rats.

SUMMARY

1. The number of days required by young male rats to grow from 60 to 200 grams has been determined in the case of 67 animals fed on a special growth diet devised by Mendel, of which 34 were injected with anterior pituitary growth hormone, and in the case of 49 animals fed on a poorer control diet, of which 27 received injections.

2. Those receiving the special diet, diet B, and the hormone, required an average of 28 days to grow from 60 to 200 grams, those receiving diet B without injection 38 days; the rats fed on the poorer diet, diet C, and administered growth hormone averaged 37 days, and the diet C controls, 50 days.

3. The results of Mendel which show that the special diet causes a more uniform growth at a more rapid rate are corroborated, and in addition it was found that injection of anterior pituitary growth hormone superimposed on the effects of the improved diet an even more rapid and uniform growth.

4. Of the diet B animals 74 per cent of the injected and only 24 per cent of the controls required 30 days or less to grow from 60 to 200 grams, while of the diet C animals 81.7 per cent of the injected rats and only 32 per cent of the controls grew to 200 grams in less than 40 days.

5. The majority of the diet B injected rats reached 200 grams in the ten day period preceding that in which the majority of the diet C animals grew to 200 grams.

6. The body length of selected numbers of all four groups regardless of how long it took them to grow from 60 to 200 grams was 195 mm. within a reasonable error.

7. At least three factors appear to be active in influencing the growth rate of the animals on the four parts of the experiment: 1, the individual growth potential of the particular rat; 2, the quality of the diet, and 3, the injection of the growth hormone. The rapidity of growth in the injected animals was limited in the majority of cases by the diet offered to the animals and to a lesser degree by the growth potential of the rat under discussion.

8. The data at hand indicate that the diet B injected animals approach the maximum average daily increase in weight of which an adolescent male rat is capable, namely, 6 to 7 grams.

In conclusion we wish to express our indebtedness to Dr. Harvey Cushing under whose direction this investigation was carried out. We wish also to thank Dr. Lafayette B. Mendel for kindly reading the paper and offering valuable suggestions.

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BLOOD REGENERATION STUDIES

II. Observations on the Blood of Normal Dogs with Special Reference to the Measurement of Volume, Erythrocytes, Leucocytes and Nitrogenous Constituents[1]

JANE M. LEICHSENRING, ALICE BIESTER, HORTENSE H. HÖNIG, SPARKLE M. FURNAS, EDITH S. FOSS and M. VIRGINIA ROUTT

From the Division of Home Economics, University of Minnesota, University Farm

Received for publication October 8, 1931

In experiments with dogs involving a study of the changes occurring in the blood, it is essential that information as to the normal range of the various blood constituents be available. Few reports have appeared in the literature which give fairly complete information on large groups of animals. It therefore has seemed desirable to present the data secured in this study which includes physical and chemical measurements on the blood of 32 adult dogs, 16 males and 16 females.

Physical measurement. For all observations reported in this paper, blood samples were secured in the morning before the dogs were fed. Blood volume was determined by the dye method of Keith, Rowntree and Geraghty (1915) using essentially the technic of Whipple and his collaborators (1920). Red cell counts and red cell diameter measurements were made in accordance with the methods described in an earlier paper (1931). The data obtained on the male and female dogs studied are presented in table 1. The averages shown for all measurements are true weighted averages, representing in each case from 52 to 120 duplicate or triplicate determinations. A comparison of the weighted averages for the males and the females indicates that no significant differences exist between the two sexes.

Although the averages for the two sexes are very similar, individual differences are relatively large. For example, total blood volume ranged from 5.90 to 9.65 cc. per 100 grams body weight for the females and from 6.03 to 8.72 cc. per 100 grams body weight for the males. Similarly, red cells in millions per cubic millimeter of blood ranged from 6.24 to 8.36 for the females and from 5.09 to 8.55 for the males. These individual differences suggest the importance of securing normal values for each animal used in a study in which changes in the blood picture are followed.

[1] Paper no. 1049 of the Journal Series of the University of Minnesota Agricultural Experiment Station.

TABLE 1

Individual and weighted averages of physical measurements on the blood of 32 dogs

DOG NUMBER	WEIGHT	BLOOD VOLUME PER 100 GRAMS	CELL VOLUME PER 100 GRAMS	PLASMA	RED CELL DIAMETER	RED BLOOD CELLS PER CU. MM.	WHITE BLOOD CELLS PER CU. MM.
Females							
	kgm.	*cc.*	*cc.*	*per cent*	*μ*	*millions*	*thousands*
16	9.98	7.19	3.76	47.4		8.36	5.55
15	14.09	6.82	3.06	55.2		7.02	3.85
20	17.56	7.17	2.98	58.5		6.24	7.72
21	11.19	6.74	3.03	55.0		7.03	9.37
22	12.40	5.90	2.66	54.9		6.77	7.30
19	17.92	7.11	4.20	41.0	6.85	7.27	4.07
32	16.33	7.16	3.44	51.9	7.15	6.78	8.51
25	10.50	6.88	3.15	54.2		6.44	8.70
26	14.73	6.58	2.88	56.3		6.66	19.33
29	17.46	6.93	3.37	51.3	6.95	6.62	13.05
1	16.29	6.94	3.33	51.9		7.72	6.04
2	12.02	7.79	3.82	50.0		8.07	
3	13.15	6.65	3.15	52.8		7.99	
4	11.91	6.28	2.36	62.3			8.95
5	9.07	9.65	4.06	58.0	7.13		
6	19.00	7.60	4.08	45.8			
Weighted average for females.....	14.42	7.06	3.35	52.5	7.05	7.17	9.09
Males							
14	23.02	8.68	5.15	40.7	6.80	7.57	6.63
16	16.56	7.34	3.61	51.9	7.10	7.89	5.30
18	18.98	6.03	2.77	54.1	6.90	6.65	5.00
27	19.05	6.82	3.78	44.4	7.10	7.03	8.38
28	20.64	8.72	5.27	39.6	6.80	7.72	4.63
30	16.90	7.61	3.07	59.7	6.98	6.54	10.03
31	20.34	7.76	3.60	53.7	7.21	6.56	9.81
33	25.25	6.70	2.82	58.0	6.96	6.18	7.22
34	22.23	7.13	3.33	53.3	6.89	6.66	9.82
35	14.35	7.49	3.51	52.4	7.01	6.76	10.33
7	15.38	7.90	3.94	50.2		8.55	3.93
8	14.40	6.60	3.13	52.6		7.76	5.50
9	12.70	6.66	2.59	61.1	7.02	5.09	7.30
10	15.88	6.71	3.58	46.6	6.98	7.01	16.17
11	12.70	8.54	3.36	60.7	7.13	5.38	
12	18.37	8.12	3.62	55.2		7.64	
Weighted average for males.....	17.24	7.30	3.52	52.0	7.01	7.17	8.49
Weighted average for all dogs.....	15.76	7.18	3.43	52.3	7.02	7.17	8.76

A comparison was made between the data secured in the present study and that reported by other investigators on normal dogs. On the basis of body weight, the total blood volume observed in this series of animals, 7.18 cc. per 100 grams body weight, was lower in general than that reported by others using the same method. Hooper, Smith, Belt and Whipple (1920) reported an average total blood volume of 10.13 cc. per 100 grams body weight in a series of 22 animals, using vital red as the dye. Harris (1920) obtained an average of 8.43 cc.[2] per 100 grams body weight, when he used vital red dye and 7.20 cc.[2] when he used congo red. This latter figure is practically identical with that reported in the present study. A very much higher figure, 11.28 cc., was reported by Powers, Bowie and Howard (1930) who used congo red as the dye in a series of 25 dogs. Our results appear to be intermediate between those reported by investigators using the carbon monoxide method and those using the direct or Welcker method. Using the carbon monoxide method, Plesch (1909) reports 7.93 cc.,[2] Gréhant and Quinquad (1882), 8.20 cc., and Arnold, Carrier, Smith and Whipple (1921), 7.78 cc. per 100 grams body weight. Brodin, Richet and St. Girons (1919–1920) and Harris (1920) using the direct method report 6.43 cc.[2] and 6.63 cc.[2] per 100 grams body weight respectively.

The plasma per cent observed in our animals, 52.3 per cent, was somewhat higher than that reported by Hooper, Smith, Belt and Whipple, 48.4 per cent, and lower than that of Powers, Bowie and Howard, 54.8 per cent. The latter suggest that their somewhat higher figures may be explained by the fact that their hematocrit tubes were centrifuged longer and at a higher rate of speed than those of other investigators. The slightly higher red cell count of 7.17 million per cubic millimeter of blood observed in the present study as compared with 7.00 million reported by Powers and coworkers suggests that the difference observed is due to an actually greater volume of cells. The average size of the red blood cells of the animals in this study was 7.0 microns. Burnett's (1917) summary of the work of six investigators shows that their figures ranged from 6.95 to 7.3 microns. Kohanawa (1928) reports a slightly lower figure, 6.8 microns. These differences can probably be explained at least in part by the methods used in making the measurements.

Considerable variation was noted in the leucocyte counts of the different animals. For the females the range was from 3.85 to 19.33 thousands per cubic millimeter of blood and for the males, 3.93 to 16.17 thousands. The average for the group, 8.76 thousands, agrees well with the average normal figure, 8.00 thousands, suggested by Burnett. The average reported by Kohanawa of 17.05 thousands would appear to be unusually high.

[2] These figures have been converted from percentage or weight of blood in terms of body weight to volume of blood in terms of body weight. The specific gravity of blood was taken as 1.056 (Sherrington and Copeman, 1893).

Chemical measurements. These determinations were made on the same series of animals reported above. Attention was centered entirely on the protein and non-protein nitrogenous constituents of the blood. Analyses on whole blood included total protein and hemoglobin. For hemoglobin the volumetric oxygen capacity method described by Van Slyke (1917) and modified by Van Slyke and Neill (1924) and for total protein the micro-Kjeldahl method of Folin and Wu (1919) using the apparatus recommended by Parnas and Wagner (1921) with the modification suggested by Pregl (1924) were employed. Non-hemoglobin protein was determined by difference. Determinations on the blood filtrate, which was prepared according to Folin's (1922) method for laked blood, included measurements of the non-protein nitrogen by the micro-Kjeldahl method, urea by Beattie's (1928) colorimetric method, pre-formed creatinine by Folin and Wu's (1919) colorimetric method, and amino acid nitrogen by the Van Slyke (1913) gasometric method.

The data secured are presented in table 2. Again the averages shown for all measurements are true weighted averages and represent in all cases, except creatinine and amino acid nitrogen, the average of 50 to 120 duplicate or triplicate determinations. A comparison of the data for the males and females showed no significant differences which could be attributed to sex. It will be noted that relatively large individual differences exist. Total protein ranged from 17.59 to 21.26 grams per 100 cc. of blood for the females and from 17.81 to 23.08 grams for the males. A much greater variation was observed in hemoglobin values. For the females the range was 9.82 to 17.11 grams per 100 cc. blood and for the males 9.77 to 16.21 grams. Similar variations were observed in non-hemoglobin protein, non-protein nitrogen, urea and amino acid nitrogen. Of the various constituents measured creatinine appeared to be the most constant in amount.

No data on the total protein and non-hemoglobin protein content of whole dog's blood were located in the literature, although considerable data are available on plasma and serum proteins. The average hemoglobin content of the blood for this series of animals was considerably lower than that reported by Plesch of 17.01 grams[3] per 100 cc. blood and also very much less than that reported by Whipple, Hooper and Robscheit. For the sake of comparison, an average was made of pre-bleeding figures on the 23 dogs reported in two of their papers (1920a, b). Calculated in terms of grams of hemoglobin per 100 cc. blood, using 13.8 grams as 100 per cent, their animals averaged 17.71 grams as compared with 14.11 grams for the animals in the present study. This difference can probably be explained by the fact that their animals were raised in their own laboratory and were constantly on a controlled diet, whereas our animals were

[3] This figure has been converted from hemoglobin content of blood in per cent to grams of hemoglobin per 100 cc. blood.

TABLE 2

Individual and weighted averages of chemical measurements on the blood of 28 dogs

DOG NUMBER	WEIGHT	TOTAL PROTEIN PER 100 CC.	HEMOGLOBIN PER 100 CC.	NON-HEMOGLOBIN PROTEIN PER 100 CC.	NON-PROTEIN NITROGEN PER 100 CC.	UREA NITROGEN PER 100 CC.	CREATININE PER 100 CC.	AMINO ACID NITROGEN PER 100 CC.
				Females				
	kgm.	*grams*	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
16	9.98	19.59	11.08	9.69	48.0			
15	14.09	17.59	13.68	3.91	39.0			
20	17.56	17.61	9.82	7.36	30.0			
21	11.19	19.37	10.55	8.81	30.0			
22	12.40	18.40	11.70	6.71	39.0			
19	17.92	21.26	17.11	4.15	35.0	16.68	1.70	4.05
32	16.33	19.93	13.32	6.31	39.0	9.12		
25	10.50	19.65	11.65	8.00	32.0			
26	14.73	18.59	11.34	7.26	41.0			
29	17.46	19.37	14.09	4.96	33.0	17.89	1.55	13.97
1	16.29		15.83					
2	12.02		16.14					
3	13.15		15.93					
Weighted average for females.....		19.24	14.11	6.70	36.0	14.02	1.62	10.66
				Males				
14	23.02	23.08	16.21	6.87	30.0	6.13	1.39	
17	16.56	20.95	13.86	6.22	29.0	9.67	1.47	4.84
18	18.98	19.42	12.96	7.52	29.0	10.33	1.60	
27	19.05	20.06	14.49	5.28	35.0	14.93	1.47	12.22
28	20.64	22.58	17.95	4.63	24.0	8.31	1.40	16.84
30	16.90	18.90	13.02	4.29	43.0	7.78		
31	20.34	20.21	12.26	7.96	41.0	9.60		
33	25.25	17.81	9.82	8.23	34.0	13.78		
34	22.23	19.24	12.72	6.52	34.0	12.32		
35	14.35	19.07	12.12	6.96	41.0	9.38		
7	15.38		15.99					
8	14.40		14.93					
9	12.70	18.19	9.77	8.42	36.0			
10	15.88	21.26	15.99	5.26	35.0			3.37
12	18.37		15.09					
Weighted average for males.....		19.93	14.11	6.59	35.0	10.31	1.49	10.82
Weighted average for all dogs.....		19.64	14.11	6.64	35.4	11.04	1.52	10.77

secured from the dog pound and therefore no knowledge of their previous history was available. The dogs in the present study, however, would seem to be more nearly representative of the animals available for most research workers. Powers, Bowie and Howard report an average hemoglobin per cent of 92.8, which, when calculated as above, would give 12.81 grams per 100 cc. blood.

A comparison of the data in the present study with that reported by Haden and Orr (1925) on the non-protein nitrogenous constituents of the blood shows a fairly close agreement. They report in a series of 200 dogs, non-protein nitrogen, 30.8 mgm.; urea nitrogen, 11.7 mgm.; creatinine, 1.5 mgm.; and amino acid nitrogen, 6.7 mgm. per 100 cc. blood, as compared with non-protein nitrogen, 35.4 mgm.; urea nitrogen, 11.04 mgm.; creatinine, 1.52 mgm.; and amino acid nitrogen, 10.77 mgm. in the present study. The results of Morgulis and Edwards (1924) on 6 dogs show a close agreement with those in table 2. They report non-protein nitrogen, 34.8 mgm.; urea nitrogen, 12.9 mgm.; creatinine, 1.6 mgm.; and amino acid nitrogen, 8.7 mgm. per 100 cc. blood.

SUMMARY

Observations on the blood of 32 normal dogs, 16 males and 16 females are reported.

The weighted average for total blood volume is 7.18 cc. and for cell volume, 3.43 cc. per 100 grams body weight; plasma per cent, 52.3; red cell count, 7.17 millions per cubic millimeter; red cell diameter, 7.0 microns; white cell count, 8.76 thousands per cubic millimeter of blood.

The weighted average per 100 cc. of blood for total protein is 19.64 grams; for hemoglobin, 14.11 grams; for non-hemoglobin protein, 6.64 grams; for non-protein nitrogen, 35.4 mgm.; urea nitrogen, 11.04 mgm.; creatinine, 1.52 mgm.; amino acid nitrogen, 10.77 mgm.

No significant sex differences were observed in any of the physical or chemical measurements reported.

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STUDIES ON CONDITIONS OF ACTIVITY IN ENDOCRINE ORGANS

XXVIII. Some Effects of Sympathin on the Nictitating Membrane

A. ROSENBLUETH[1] AND W. B. CANNON

From the Laboratories of Physiology in the Harvard Medical School

Received for publication September 24, 1931

In previous studies of sympathin (Cannon and Bacq, 1931; Rosenblueth and Schlossberg, 1931), the non-anesthetized animal with spinal cord transected or the animal with brain destroyed was used. Acceleration of the denervated heart and an increase of the low blood pressure of the pithed animal have been the chief indicators—the former involving a complicated and difficult technique, the latter involving operative hazards. Distinct advances would be made, therefore, if an anesthetic which would not interfere with the action of sympathin could be devised. It would be interesting, also, to know whether gland cells (e. g., those of the liver) as well as smooth muscle, when subjected to sympathetic stimulation, give off sympathin, and whether sympathin and adrenin, which under natural conditions would be produced simultaneously, are synergistic. These and other questions we hoped to answer in undertaking this research.

Since sympathin acts like adrenin we thought we might find in the smooth muscle of the nictitating membrane (cf. Querido, 1924) a simple indicator. We fixed firmly in a Czermak holder the head of a cat, turned the head so that the nictitating membrane (n. m.) would stretch upward vertically, and attached to its edge by means of a vertical thread and light spring clip the short end of a writing lever. Thus contraction of the membrane was registered, commonly with seven-to-ten-fold magnification. The membrane of the right side was always used. Invariably its sympathetic nerve supply was abolished,—by severance of the cervical strand in a few acute experiments, by previous removal of the superior cervical ganglion in all others.

We performed two series of experiments, a short series of preliminary tests in animals with brain pithed transversely (cf. Cannon and Britton, 1925), and a second series under anesthesia.

Results. *The nictitating membrane can be used as a quantitative indi-*

[1] John Simon Guggenheim Fellow in Physiology, from Mexico.

cator. This point was proved first in an acute experiment by injecting into a vein, repeatedly and at the same rate, the same dose of adrenalin, and finding that the successive records of the contracting membrane almost exactly coincided. The coincidence was quite as satisfactory as in the blood-pressure test devised by Elliott (1912). The contraction of the membrane varied in correspondence with the size of the dose of adrenalin.

In figure 1 are presented the records of the contraction of the right n. m. of a cat prepared four days previously by removal of the right superior cervical sympathetic ganglion (supr. cerv. symp. g.), and the left adrenal gland. In this experiment the animal was decerebrated, and electrodes were placed on the right splanchnics. As the records show, adrenin secreted in response to splanchnic stimulation had effects corresponding to the position of the secondary coil. (A Harvard inductorium was used,

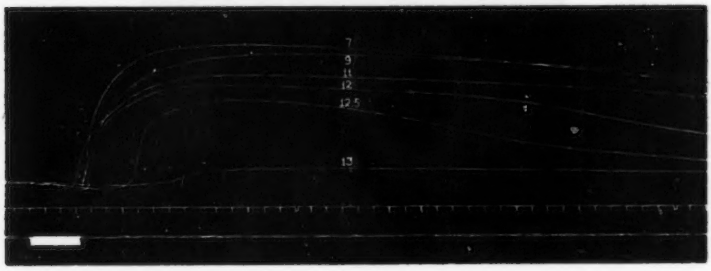

Fig. 1. Records of contraction of the nictitating membrane (disconnected from the superior cervical ganglion four days previously), when stimulated by adrenin secreted in response to different intensities of splanchnic stimulation for 15 seconds each. The numerals indicate in centimeters the distance of the secondary coil (Harvard inductorium). The order of stimulation was 11, 12, 13, 9, 7, 12.5. Primary current, 10 volts. In this and other records time was marked in 5-second intervals.

with distance marked in centimeters.) A primary current of 10 volts was always used.

The nictitating membrane is sensitized to adrenin and sympathin by previous denervation and by cocaine. In an acute experiment on a cat weighing 3.5 k. we found that after the injection of 25 mgm. of cocaine hydrochloride, approximately the same extent of contraction was caused by 1 cc. of adrenalin, 1:1,000,000, as had been caused before by 1 cc. of adrenalin, 1:100,000, injected at the same rate. We found similar sensitization to secreted adrenin.

Although adrenin discharged by splanchnic stimulation can cause the freshly denervated n. m. to contract, the effect is relatively slight. It is much greater if the supr. serv. symp. g. connected with the membrane has been removed at least 4 or 5 days before the experiment. And even this

larger contraction can be increased by intravenous injection of cocaine (usually from 6 to 10 mgm. per k., injected slowly). In an experiment on a decerebrate animal, whose right supr. cerv. symp. g. had been removed 6 days before, the right n. m. responded to secreted adrenin with an almost maximal contraction. After cocaine, splanchnic stimulation of the same intensity and duration as before caused a 16 per cent increase in the height of contraction and more than doubled its duration. (In this instance the effect of splanchnic stimulation for 30 seconds (coil distance 8 cm.) was closely matched by 0.5 cc. adrenalin, 1:50,000, injected into a femoral vein during 10 seconds.) Evidently sensitization by cocaine is independ-

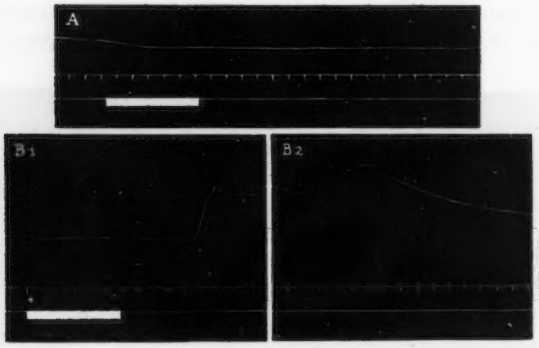

Fig. 2, A. Absence of effect of sympathin on the nictitating membrane sensitized by removal of its sympathetic ganglion. The pilomotor muscles of the tail were maximally contracted by stimulation of the isolated lower abdominal sympathetic chains for 30 seconds (at 3:47).

B_1. The beginning of the contraction of the membrane after the same stimulation (at 4:07) as in A (injection of 19 mgm. of cocaine completed at 4:01; weight of animal 4.2 k.).

B_2 The end of the contraction (at 4:15) that started at 4:07.

ent of sensitization by sympathetic ganglionectomy, and their effects may sum.

Sympathin was produced first by the procedure used in earlier experiments, i.e., by such stimulation of the isolated lower abdominal sympathetic chains (l. a. s.) as caused contraction of the pilomotor muscles of the tail. Without sensitization by cocaine we never produced contraction of the n.m. by sympathin, though the membrane had been sensitized by previous excision of its superior cervical ganglion. After cocaine, however, we have succeeded in making even the freshly denervated membrane contract, but only to a slight degree. If the ganglion has been removed several days before the experiment, stimulation of the l. a. s., after the mem-

brane has been sensitized by cocaine, always results in a marked contraction. The records in figure 2 are taken from an animal in which the n. m. had been denervated (i.e., the superior cervical ganglion removed) 6 days before, and which was decerebrated. Stimulation of the l.a.s. before cocaine for 30 seconds (fig. 2, A) had no effect; after cocaine the same stimulation caused, after the usual long latent period, a typical contraction which continued high and ended in about eight minutes (fig. 2, B_1 and B_2).

"Dial" anesthesia does not interfere with the response of the nictitating membrane to adrenin and sympathin. The results thus far reported were secured on animals with brain pithed. Occasional loss of prepared animals made desirable a method of experimenting free from that hazard. We tried ether anesthesia but found that it caused contraction of the n. m., greater the deeper the anesthesia, and that then the effect of sympathin was much reduced. Experience in the Laboratory had proved that dial, "Ciba" produced anesthesia with remarkably little disturbance of a number of physiological processes, and we therefore tried it. To our great satisfaction it did not interfere at all with the effects of sympathin which we were studying. Since we wished to avoid so far as possible exciting the animal, we gave the dial at first subcutaneously or intramuscularly. Later we learned that we could give it intraperitoneally without affecting the nerves there, and our final method was to inject it, in a dose of 0.7 cc. per k., into the peritoneal cavity about twenty minutes before we expected to start the experiment. That dose produced complete anesthesia.

Sympathin is produced when occasionally contracted smooth muscle, and also when tonically contracted smooth muscle, is stimulated. In the original observations on sympathin Cannon and Baeq (1931) stimulated the sympathetic fibers distributed to the smooth muscles of the tail and the intestines, i.e., those of the hairs and the intestinal wall, which are occasionally affected by sympathetic impulses, and those of the blood vessels of the two regions, that are tonically affected. It was assumed that the sympathin was given off from the smooth muscle of the blood vessels as well as from the other smooth muscle. Repeated stimulation commonly produced diminishing effects. Quite possibly this was due to the exhaustion of a store of the substance. Might it not be true, therefore, that the store was held in the muscles occasionally affected, and that tonically active muscles would not have an extra amount which could be set free? In order to answer this question it would be necessary to stimulate the two types of muscle separately.

In confirmation of the earlier observations we stimulated the l. a. s. after cutting the connections to the tail, severing the sciatic nerves and excising the inferior mesenteric ganglion. The only effect then was an erection of the hairs over a small region on the rump, where blood vessels are few. Figure 3, A shows the contraction of the n. m. (sensitized by

being denervated eight days before and by cocaine) which resulted from stimulation of the muscles of the rump hairs. This patch of skin was

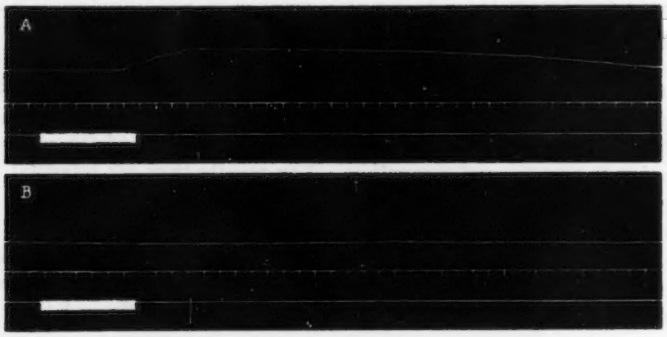

Fig. 3, A. Contraction of the sensitized nictitating membrane on stimulating exclusively the sympathetic nerves to smooth muscles of the hairs in a patch of skin on the rump. Dial anesthesia.

B. Absence of the effect when the same stimulus (coil distance 8 cm. 30 seconds) was repeated, after removal of the patch of skin.

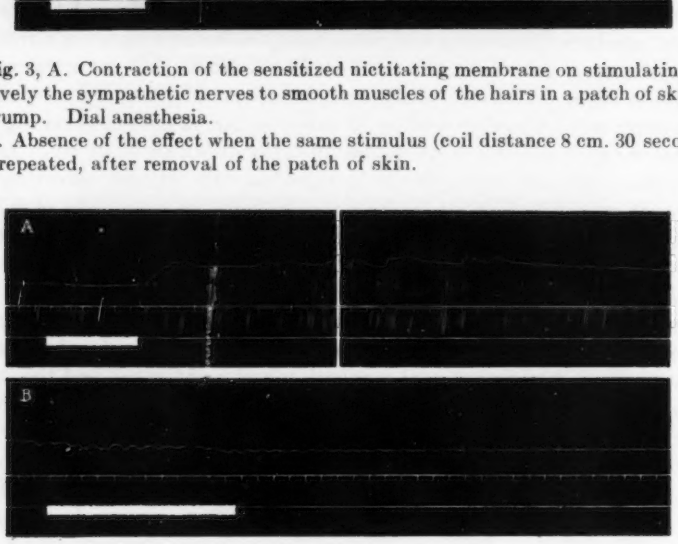

Fig. 4, A. Beginning and end of contraction of the sensitized nictitating membrane on stimulating exclusively (30 seconds) the sympathetic nerves to the hind legs (no erection of hairs). Two minutes of record omitted. The oscillations were due to spontaneous contractions of the membrane. Dial anesthesia.

B. Absence of the effect when, after severance of the sciatic nerves the same stimulus (coil distance 6 cm.) was applied for 60 seconds.

now cut away. Repetition of the stimulus had no effect, as shown in figure 3, B.

To test the effect of stimulating tonically contracted smooth muscle we cut the connections of the l. a. s. except those to the legs and rump,

removed the skin of the rump which was responding with erect hairs, and then stimulated the l. a. s. for 60 seconds, with the secondary coil at 6 cm. The nictitating membrane, denervated ten days before and sensitized by cocaine, contracted after a latent period of about 75 seconds (see fig. 4, A). The contraction lasted about 5 minutes. The sciatic nerves were then severed and the stimulation was exactly repeated. There was no response (see fig. 4, B). Two other experiments bearing on the same question confirmed these results. It appears, therefore, that sympathin is given off from the smooth muscle (and possibly other cells) of blood vessels, that are held in tonic contraction by sympathetic impulses. The only other structures, apparently, which might be involved are sweat glands, and in the cat they would play a very minor rôle.

The amount of sympathin produced by smooth muscle depends on the size of the area stimulated. Although this point seemed self-evident we thought it should be demonstrated, in order to account for occasional negative effects.

Figure 1 shows that the n. m. can be used as a quantitative indicator of adrenin. Since in all respects now known sympathin acts like adrenin, we may reasonably assume that the n. m. would serve also as a quantitative indicator of sympathin. The slight contraction of the n. m. when a small patch of hairs on the rump was affected (fig. 3, A) as compared with the much larger and longer contraction when the smooth muscle of the blood vessels and hairs of the whole tail region was stimulated (fig. 2, B_1 and B_2) illustrates the importance of the area involved, but only roughly, because the records were not taken from the same animal under the same conditions.

Decisive results were obtained in an animal with a n. m. deprived of its ganglion ten days before, and sensitized by cocaine as well, and with the adrenals tied off. Electrodes on a branch of the superior mesenteric artery allowed stimulation of its attendant nerves for 30 seconds (with coil distance 6 cm.). There was no effect on the membrane. The electrodes were at once transferred to the main trunk of the artery, and its attendant nerves were stimulated for 30 seconds, as before. A well marked contraction of the n. m. resulted. In other preparations, likewise, we had no difficulty in producing contraction of the membrane when we stimulated as large an area of smooth muscle as is involved in the distribution of the superior mesenteric artery or the duodeno-hepatic artery with the hepatic fibers cut. When a smaller area is stimulated we must assume that sympathin is liberated, but in an amount too small to be effective.

Sympathin and adrenin act cöoperatively. As is now well known, conditions which cause widespread discharge of sympathetic nerve impulses (e.g., excitement, cold, asphyxia) cause simultaneously discharge of adrenin into the blood stream. But sympathetic impulses (e.g., in excite-

ment) also set free sympathin (Newton, Zwemer and Cannon, 1931), which circulates in the blood and which, so far as now known, acts like adrenin. In previous sections of this paper we have shown that the n .m. responds quantitatively to different concentrations of adrenin and sympathin. By selecting minimal or weak stimuli of splanchnic and l. a. s. nerves we were able to call forth a small amount of adrenin and of sympathin respectively, and then, by simultaneous stimulation of the two sets of nerves, we could determine whether adrenin and sympathin are synergistic.

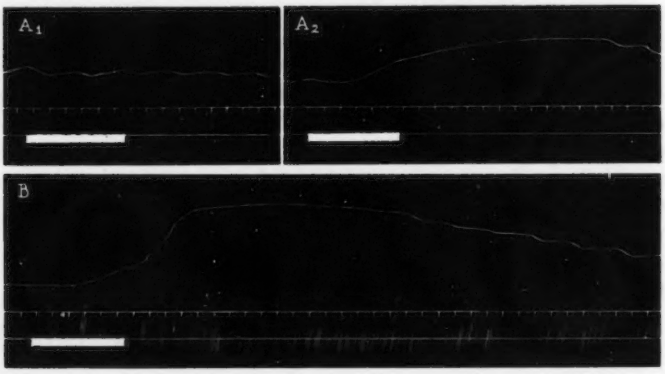

Fig. 5, A_1. Absence of contraction of the sensitized nictitating membrane on stimulating the isolated splanchnic nerves, with coil distance 10.5 cm. Dial anesthesia.

A_2. Slight contraction of the membrane when the lower abdominal sympathetic strands were stimulated, with coil distance 7 cm.

B. Contraction of the membrane when the two sets of nerves were stimulated simultaneously, with the same strength and duration of stimuli as in the separate stimulations.

A typical result is illustrated in figure 5. The right supr. cerv. symp. g. had been removed two weeks before. The cat (weighing 3.6 k.) was anesthetized with dial and given 25 mgm. of cocaine. Shielded electrodes were placed on the right splanchnic nerves (which were crushed centrally) and on the l. a. s. strands (which were cut above). Each pair of electrodes was connected to a Harvard inductorium. Repeatedly stimulation of the splanchnics for 30 seconds, with the secondary coil at 10.5 cm., had no effect on the n. m. (see fig. 5 A_1), although with the coil at 7 the same length of stimulation caused a maximal contraction. When the l. a. s. strands were stimulated, with the secondary at 7 cm. the tail hairs were erected, but the n. m. contracted only slightly (see fig. 5, A_2). Stimulation of the splanchnics and the l. a. s. strands at the same time invariably produced a

large contraction, much higher and longer than the separate stimulations would lead one to expect (see fig. 5, B).

The results recorded in figure 5 were confirmed in other animals with modifications of the two factors—by adjusting the currents so that no perceptible effect was produced by l. a. s. stimulation and only a slight effect when the splanchnics were excited, and also by combining slight effects from the two sources. In one instance under splanchnic stimulation with coil distance 12 cm. the n. m. questionably contracted (the rise in an 8-fold magnification was 4 mm.), and under l. a. s. stimulation (6 cm.) there was no effect. When the two stimulations were applied simultaneously for the same period, the rise in the record was 21 mm., and lasted more than four minutes. It is clear that adrenin and sympathin work together in affecting structures innervated by the sympathetic system.

The liver discharges into the circulating blood a substance which acts like sympathin. In 1921, Cannon and Uridil, and later Cannon and Griffith (1922) reported that stimulation of the hepatic nerves causes promptly the discharge of a substance into the circulation that makes the denervated heart beat faster and that raises blood pressure, much as adrenin does. The feeding of protein food increased the effects; fasting or the feeding of carbohydrate or fat lessened them. The discovery of sympathin raises the interesting question whether the cardio-accelerator agent given off by the liver, when stimulated through its sympathetic nerves, is not itself sympathin.

The record reproduced in figure 6 presents a typical effect on the n. m. of stimulating the hepatic nerves. In this instance the right supr. cerv. symp. g. had been removed seven days, the n. m. had been further sensitized by cocaine (30 mgm. per 4.2 k.), the adrenal glands had been tied off *en masse*, and the duodenal artery with its nerves had been crushed in a very tight ligature. Stimulation through electrodes on the duodenohepatic nerves had, therefore, effects only on the liver.

In the record reproduced in figure 6, as well as in other records from other animals, the quick, high contraction, quite like that caused by adrenin when the splanchnics are stimulated, is wholly characteristic. The usual long latent period, which is typical of the contraction of the n. m. when smooth muscle of the hairs or blood vessels is stimulated (see figs. 2, 3 and 4), is not seen when the hepatic nerves are stimulated. It would appear, therefore, that the stubstance given off from the stimulated liver comes from the liver cells and not from the smooth muscle of the blood vessels, or not from that source alone. In this connection it is interesting to note that the typical effect was produced on stimulating the hepatic nerves in an animal in which the portal circulation had been shunted into collateral channels for ten days.

Simultaneous records of the contraction of the n. m. and the beats of the denervated heart, in the same animal, showed that the hepatic substance

has effects which correspond both in their course of development and their intensity, on these two indicators. And when the l. a. s. strands and the duodeno-hepatic nerves were stimulated at the same time, each with a sub-maximal current, the n. m. contracted more than when each was stimulated separately with the same current—i.e., the hepatic substance and sympathin coöperated. In short, the only differences between the substance and sympathin that appeared were quantitative.

Although the foregoing observations strongly suggest that the hepatic cardio-accelerator agent described by Cannon and Uridil is really sympathin, we cannot conclude from present evidence that they are identical. As noted above, the hepatic substance has much greater effects if the animal is digesting protein. And yet Newton, Zwemer and Cannon (1931) found that excitement (causing a discharge of sympathin) had as much effect in a fasting animal as in the same animal when well fed with meat.

Fig. 6. Contraction of the sensitized nictitating membrane on stimulating the nerves to the liver (30 seconds, coil distance 5).

Furthermore, they observed quite regularly a considerable period between the beginning of excitement and the maximal increase of the rate of the denervated heart—a period of about three minutes. This corresponded quite closely with the observations of Cannon and Bacq (1931), and is in accord with the results reported in the present paper, on the action of sympathin produced by stimulating smooth muscle. Newton, Zwemer and Cannon never found a quick, large acceleration of the rate of the denervated heart, except when the adrenal glands were active. That stimulation of the hepatic nerves has practically the same effect as secreted adrenin (in the anesthetized animal) is a curious fact. It may have important bearings on the formation of sympathin and also of adrenin in the body. Further evidence as to the nature of the hepatic substance must be sought in future investigations.

The spontaneous oscillations in the records of the nictitating membrane. It is noteworthy that at times contraction of the n.m. occurs and runs its

full length to complete relaxation with a smooth curve (cf. fig. 6). On the other hand, not uncommonly there appear at an intermediary stage in the relaxation of the contracted membrane rhythmically recurring small contractions, having in different animals various periods and forms (cf. figs. 2, 4 and 5). In a few preparations these spontaneous waves are constantly recurring and disappear only during a stimulated contraction of the membrane (cf. fig. 5). Rarely they are so large as to prevent satisfactory records. Their existence seems to be favored by light anesthesia. We have found that changing the weight of the writing lever will sometimes reduce, if not abolish, the waves. They should be carefully distinguished from disturbances of the record due to oscillations of the eyeball or deep respiratory movements. Their cause probably lies deep in the mystery of the physiology of smooth muscle.

SUMMARY

A method is described by which the smooth muscle in the nictitating membrane, sensitized by being previously disconnected from the superior cervical ganglion and by action of cocaine, can be used to record the presence of adrenin and sympathin in the circulating blood (see fig. 2).

The membrane can be used as a quantitative indicator (see fig. 1).

"Dial" as an anesthetic does not interfere with the action of adrenin and sympathin on the nictitating membrane.

Sympathin is produced when occasionally contracted smooth muscle (e.g., of the hairs), and also when tonically contracted smooth muscle (e.g., of the blood vessels), are stimulated by sympathetic impulses (see figs. 3 and 4).

The amount of sympathin produced on stimulation of smooth muscle, when circumstances are comparable, depends on the size of the area stimulated.

Sympathin and adrenin liberated into the circulation at the same time act coöperatively (see fig. 5).

The liver discharges into the circulating blood a substance which acts like sympathin (see fig. 6). The relation between this substance and the hepatic cardio-accelerator agent described by Cannon and Uridil (1921), and the question of its identity with sympathin, are considered.

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ON GLYCOLYSIS AND OXIDATION OF SUGAR IN BLOOD, INCUBATED AND CIRCULATING "IN VITRO"

E. W. H. CRUICKSHANK AND C. W. STARTUP

From the Department of Physiology, Dalhousie University, Halifax, N. S.

Received for publication August 10, 1931

In the study of the carbohydrate metabolism of the isolated heart-lung preparation we found, at times, a more or less marked irregularity in the amount of sugar disappearing from the samples of defibrinated blood placed in the incubator. Further, in order to correct our results for the amount of sugar that had been removed by the tissue we estimated the lactic acid of the blood in circulation as well as of that in the incubator, and again we encountered irregularities. We therefore decided to investigate the question of the distribution and disappearance of sugar and lactic acid in heparinised and defibrinated blood, in circulation in an artificial schema and in the incubator.

METHODS. *Sugar and lactic acid estimation.* Sugar was estimated by the Shaffer, Hartmann method (1920) and lactic acid by West's modification of the method described by Friedemann, Cotonio and Shaffer (1927) and by Friedemann and Kendall (1929). It was found that, in order to secure absolutely comparable results for lactic acid, the amount of water used for washing, and the amount of bicarbonate solution used in the course of the estimation had to be kept constant.

The circulatory and oxygenation system. Six hundred cubic centimeters of dog's blood were circulated through an oxygenator by means of a pump of the pattern described by Dale and Schuster (1927). The oxygenator was a slightly modified form of that described by Bayliss, Fee, and Ogden (1928).

Oxygen estimations were made but the device used proved not to be sufficiently accurate to allow of any deductions being made from them. Numerous experiments have been carried out on incubated blood: three complete experiments on the same blood in circulation through the oxygenator and in the incubator. Because of the length of the protocols only one can here be given.

RESULTS. *The distribution of sugar and lactic acid.* To determine the distribution of sugar and lactic acid between blood plasma or serum and corpuscles, the blood was rapidly centrifuged, the ratio of cells to plasma noted and the corpuscular content determined by difference. The direct

determination of sugar and lactic acid in the cell is useless because of the diffusion of these substances from the cell due to washing, and the alteration in the normal trend of oxidation and glycolysis within the cell.

1. *In circulating blood.* A comparison of tables 1 and 2 will show two noteworthy points; *first*, defibrination causes a greater disturbance of the sugar equilibrium between plasma and cells than is occasioned by the use of heparine: *second*, the lactic acid content of the blood is markedly increased when the blood is defibrinated. When heparine is added to the

TABLE 1

Percentage content and distribution of sugar and lactic acid in whole blood, plasma and corpuscles

TIME		CIRCULATING BLOOD: WITH HEPARINE			
		Sugar	Distribution	Lactic acid	Distribution
hours		*grams per cent*		*mgm. per cent*	
0	Blood	0.0969	100	56.70	100
	Plasma	0.0636	65.60	43.97	77.50
	Cells	0.0333	34.40	12.73	22.50
1	Blood	0.0854	100	56.70	100
	Plasma	0.0595	69.67	44.51	79.60
	Cells	0.0259	30.33	12.19	21.40
2	Blood	0.0741	100	56.70	100
	Plasma	0.0526	70.80	45.17	79.60
	Cells	0.0215	29.20	11.53	20.40
3	Blood	0.0602	100	58.50	100
	Plasma	0.0443	73.10	46.38	79.30
	Cells	0.0159	26.90	11.12	20.70

TABLE 2

Percentage content and distribution of sugar and lactic acid in whole blood, serum and corpuscles

TIME		CIRCULATING BLOOD: DEFIBRINATED BLOOD			
		Sugar	Distribution	Lactic acid	Distribution
hours		*grams per cent*		*mgm. per cent*	
0	Blood	0.0764	100	76.50	100
	Serum	0.0621	81.1	54.45	71.2
	Cells	0.0143	18.9	22.05	28.8
1	Blood	0.0663	100	83.70	100
	Serum	0.0551	83.9	61.63	73.6
	Cells	0.0106	17.1	22.07	26.4
2	Blood	0.0555	100	90.00	100
	Serum	0.0411	84.9	67.16	74.6
	Cells	0.0084	15.1	22.84	25.4
3	Blood	0.04401	100	97.47	100
	Serum	0.03747	89.2	74.20	79.2
	Cells	0.00654	10.8	23.27	20.8

blood the lactic acid content is maintained almost at a constant figure both in the cell and plasma.

With agitation the blood loses most of its CO_2 liberating base which is taken up by lactic acid thus accounting for a higher lactic acid content. It will also be seen that with defibrination there is a definite alteration in the distribution of lactic acid between the serum and the cell, the serum content rising while that of the cell falls. Heparine it would seem causes little disturbance in lactic acid equilibrium.

2. *In incubated blood.* Tables 3 and 4 again demonstrate the fact that defibrination causes an alteration in the sugar ratio, somewhat similar to

that seen in the circulating blood. As was found in defibrinated blood in circulation (table 2) there is an increase in the total lactic acid of the blood. When heparine has been added to the blood there is no such change in the absolute amount of lactic acid in the plasma or the cells. It would seem therefore that as far as the maintenance of equilibrium between the sugar and lactic acid in the plasma and cells is concerned, blood treated with heparine is much more stable than that which has been defibrinated.

OXIDATION AND GLYCOLYSIS IN WHOLE BLOOD, SERUM OR PLASMA AND CORPUSCLES. A considerable amount of work has been carried out on

TABLE 3

Percentage content and distribution of sugar and lactic acid in whole blood, plasma and corpuscles

TIME		INCUBATOR BLOOD: WITH HEPARINE			
		Sugar	Distribution	Lactic acid	Distribution
hours		*grams per cent*		*mgm. per cent*	
0	Blood	0.0969	100	56.70	100
	Plasma	0.0636	67.57	43.90	77.50
	Cells	0.0333	34.43	12.73	22.50
1	Blood	0.0846	100	56.25	100
	Plasma	0.0595	70.30	43.25	79.90
	Cells	0.0251	29.70	13.00	20.10
2	Blood	0.0716	100	56.70	100
	Plasma	0.0526	73.46	45.25	79.80
	Cells	0.0190	26.54	11.45	20.20
3	Blood	0.0602	100	62.70	100
	Plasma	0.0443	73.58	48.39	77.17
	Cells	0.0159	26.42	14.31	22.83

TABLE 4

Percentage content and distribution of sugar and lactic acid in whole blood, serum and corpuscles

TIME		INCUBATOR BLOOD: DEFIBRINATED BLOOD			
		Sugar	Distribution	Lactic acid	Distribution
hours		*grams per cent*		*mgm. per cent*	
0	Blood	0.07648	100	76.50	100
	Serum	0.06209	81.1	54.45	71.40
	Cells	0.01439	18.9	22.05	28.60
1	Blood	0.06632	100	85.50	100
	Serum	0.05586	81.3	62.94	73.60
	Cells	0.01046	18.7	22.56	26.40
2	Blood	0.05850	100	93.47	100
	Serum	0.05092	87.1	70.11	75.00
	Cells	0.00758	12.9	23.36	25.00
3	Blood	0.04943	100	101.64	100
	Serum	0.04377	87.3	76.46	75.22
	Cells	0.00566	12.7	25.18	27.88

glycolysis in blood. The term glycolysis has been generally interpreted as the disappearance of sugar from the blood, no distinction being made between the breakdown of sugar to lactic acid and its complete oxidation.

Macleod (1913) has shown that the loss of sugar from incubated dog's blood, defibrinated or oxalated, at 40°C. is of the order of 28 per cent in two hours and 42 per cent in three hours, and he has stated that this sugar loss, or "glycolysis," takes place in the cell only. In later experiments upon the effect of insulin Macleod and others (1923) have further shown that the sugar lost from dog's blood to which boiled insulin had been added

is 45 per cent in two hours and above 60 per cent in three hours. Loeb (1913) has suggested that the glycolytic activity is a function of the red blood cell, through the envelope of which the sugar must pass. He supports his suggestion on the fact that sugar disappears from the serum to a greater extent than from the cell, in the blood of the dog where the cell envelope is known to be very permeable to sugar. That the leucocytes as well as the erythrocytes play a part in the removal of sugar has been demonstrated by Levene and Meyer (1912). It has also been shown by Rona and Döblin (1911) that while glycolysis can and does proceed under anaerobic conditions nevertheless the presence of oxygen favours the process. Many observers have stated that glycolysis does not take place in serum (Rona and Döblin, 1911; Van Noorden, Jr., 1912). By glycolysis again is meant merely a loss of sugar from the blood. That glycolysis *per se* will not take place in normal blood plasma and to a very limited extent in defibrinated or heparinised blood we are agreed, but that in the presence of erythrocytes which are constantly oxidising sugar and whose cell envelope is permeable to this substance, there should be no loss of sugar from the plasma in circulating blood, we can not very well believe. It will be seen from the results quoted in this paper that where there is strictly no glycolysis yet there is a loss of sugar from the plasma: this sugar is oxidised and it can, as far as we can see, only be oxidised by the cell which in the process of its oxidations must call upon the sugar in the plasma.

The extent of glycolysis in the plasma and in the cells can be determined by following the changes in the lactic acid content of both, an increase indicating glycolysis, a decrease indicating an increased oxidation of lactic acid. In such work it is essential to keep the temperature of the blood constant, for the rate and degree of glycolysis vary markedly with changes in temperature. In these experiments (tables 5, 6, 7, 8 and 9) from hourly estimations of the sugar and lactic acid content we calculated per 100 cc. of blood, the loss of sugar and the increase of decrease in lactic acid and deducted therefrom the amounts of sugar glycolysed and oxidised in blood circulating through the oxygenator and in samples of the same blood placed in the incubator. Under both conditions the effect of heparine and defibrination was studied. The blood was kept at a temperature of 36°C.

1. *In circulating blood. Sugar utilisation:* From table 5 it can be seen that heparinised blood in circulation through the oxygenator loses sugar at the rate of 12.26 mgm. per 100 cc. of blood per hour. The plasma and the corpuscles show an hourly loss of 6.45 mgm. and 5.84 mgm. respectively. When the blood obtained from the same source is defibrinated the loss for whole blood, serum and corpuscles is respectively 10.83 mgm., 8.21 mgm. and 2.62 mgm. per 100 cc. of blood per hour (table 6).

The comparative loss of sugar under these two conditions is seen in

table 9: the heparinised blood has lost 37.9 per cent, defibrinated blood 42.5 per cent, the corpuscles have lost 52.3 per cent and 55.0 per cent of their total sugar content, in heparinised and defibrinated blood respectively.

TABLE 5

Showing absolute and relative changes in sugar and lactic acid content and the relation of sugar glycolysed to that oxidised

		CIRCULATING BLOOD: WITH HEPARINE			
	Time	Fall in sugar	Increase in lactic acid	Sugar glycolysed	Sugar oxidised
Whole blood					
	hrs.	*grams per 100 cc. blood*	*mgm. per 100 cc. blood*	*grams*	*grams*
Absolute changes	1	0.01159	Nil	Nil	0.01159
	2	0.01130	Nil	Nil	0.01130
	3	0.01390	1.8	0.0009	0.01300
Per cent changes	1	11.8	Nil	Nil	100
	2	13.2	Nil	Nil	100
	3	16.2	3.2	2.2	93.5
Plasma					
Absolute changes	1	0.00415	0.54	(0.00027)	0.00388
	2	0.00690	0.66	(0.00023)	0.00657
	3	0.00830	1.21	(0.00060)	0.00770
Per cent changes	1	6.5	1.2	6.6	93.4
	2	11.6	1.4	4.8	95.2
	3	15.7	1.7	8.4	91.6
Corpuscles					
Absolute changes	1	0.00744	−0.54	(0.00027)	0.00771
	2	0.00440	−0.66	(0.00033)	0.00473
	3	0.00560	−0.41	(0.00020)	0.00580
Per cent changes	1	22.2	Nil	Nil	100+3.6
	2	16.9	Nil	Nil	100+7.5
	3	26.4	Nil	Nil	100+3.5

TABLE 6

Showing absolute and relative changes in sugar and lactic acid content and the relation of sugar glycolysed to that oxidised

		CIRCULATING BLOOD: DEFIBRINATED BLOOD			
	Time	Fall in sugar	Increase in lactic acid	Sugar glycolysed	Sugar oxidised
Whole blood					
	hrs.	*grams per 100 cc. blood*	*mgm. per 100 cc. blood*	*grams*	*grams*
Absolute changes	1	0.01016	7.20	0.00360	0.00656
	2	0.01085	6.30	0.00315	0.00770
	3	0.01149	7.46	0.00373	0.00776
Per cent changes	1	13.3	9.4	35 4	64.6
	2	16.3	7.5	29.0	71.0
	3	20.7	8.3	32.5	67.5
Serum					
Absolute changes	1	0.00641	7.18	0.00359	0.00282
	2	0.00859	5.53	0.00278	0.00581
	3	0.00963	7.04	0.00352	0.00661
Per cent changes	1	8.3	13.1	56.1	43.9
	2	12.9	8.9	67.6	67.4
	3	20.4	10.4	36.6	63.4
Corpuscles					
Absolute changes	1	0.00375	0.02	0.00001	0.00374
	2	0.00226	0.77	0.00038	0.00188
	3	0.00186	0.43	0.00021	0.00163
Per cent changes	1	26.2	0.09	0.1	99.9
	2	21.2	3.4	16.9	83.1
	3	22.1	1.88	12.5	87.5

Lactic acid: The most noteworthy fact concerning lactic acid in this type of "in vitro" experiment is that, given efficient oxygenation of circulating blood, with heparine as the anticoagulant, there is no increased formation of lactic acid either in the whole blood or in the cell (tables 6 and 9). With defibrination the results are different, there is a steady hourly increase in lactic acid in the whole blood and serum and a definite increase

in the corpuscles during the experimental period of three hours (tables 6 and 9).

Glycolysis and oxidation: A comparative study of tables 5 and 6 reveals the fact that the sugar of blood undamaged by defibrination disappears

TABLE 7

Showing absolute and relative changes in sugar and lactic acid content and the relation of sugar glycolysed to that oxidised

		INCUBATOR BLOOD: WITH HEPARINE				
	Time	Fall in sugar	Increase in lactic acid	Sugar glycolysed	Sugar oxidised
		Whole blood				
	hrs.	*grams per 100 cc. blood*	*mgm. per 100 cc. blood*	*grams*	*grams*
Absolute changes	1	0.0123	−0.45	(0.00022)	0.0123
	2	0.0130	0.45	0.00022	0.0128
	3	0.0114	6.00	0.00300	0.0840
Per cent changes	1	12.6	Nil	Nil	100+0.18
	2	15.3	0.79	9.2	90.8
	3	15.9	10.5	26.3	73.7
		Plasma			
Absolute changes	1	0.0041	−0.65	(0.00032)	0.00442
	2	0.0069	2.00	0.00100	0.00590
	3	0.0083	3.14	0.00157	0.00673
Per cent changes	1	6.4	Nil	Nil	100+7.7
	2	11.5	5.6	14.5	85.5
	3	11.5	6.9	19.2	80.8
		Corpuscles			
Absolute changes	1	0.00820	0.27	0.00013	0.00807
	2	0.00610	−1.55	(0.00077)	0.00611
	3	0.00310	2.86?	0.00143	0.00167
Per cent changes	1	24.6	2.1	1.70	98.3
	2	24.3	Nil	Nil	100+
	3	16.3	24.9?	46.1	53.9?

TABLE 8

Showing absolute and relative changes in sugar and lactic acid content and the relation of sugar glycolysed to that oxidised

		INCUBATOR BLOOD: DEFIBRINATED BLOOD				
	Time	Fall in sugar	Increase in lactic acid	Sugar glycolysed	Sugar oxidised
		Whole blood				
	hrs.	*grams per 100 cc. blood*	*mgm. per 100 cc. blood*	*grams*	*grams*
Absolute changes	1	0.01016	9.00	0.00450	0.00566
	2	0.00782	7.97	0.00398	0.00384
	3	0.00907	8.17	0.00408	0.00499
Per cent changes	1	13.2	11.7	44.2	55.8
	2	11.7	12.3	50.8	49.2
	3	15.5	8.7	44.99	55.01
		Serum			
Absolute changes	1	0.00623	8.49	0.00424	0.00199
	2	0.00815	7.17	0.00358	0.00457
	3	0.00715	6.35	0.00317	0.00398
Per cent changes	1	10.3	15.5	68.0	32.0
	2	14.4	11.3	43.9	56.1
	3	14.0	8.9	44.3	55.7
		Corpuscles			
Absolute changes	1	0.00393	0.51	0.00025	0.00368
	2	0.00288	0.80	0.00040	0.00248
	3	0.00192	1.82	0.00091	0.00101
Per cent changes	1	27.3	0.23	6.36	93.64
	2	27.5	3.5	13.80	86.20
	3	25.3	19.4	47.4	52.6

by oxidation and not by glycolysis. Heparinised blood shows no increase in lactic acid in the first two hours of the experiment and only a very slight increase of 1.8 mgm. per cent during the third hour. This shows an absence of glycolysis and a maximal oxidation of blood sugar. With plasma the oxidation is of the order of 90 and 95 per cent. Of more interest is the

sugar utilisation of the corpuscles: here it is apparent that not only does the cell oxidise its sugar but may oxidise a certain amount of its lactic acid. The amount of lactic acid disappearing is admittedly extremely small, the lactic acid figure being within the experimental error, but such a result shows that here, in the strict sense of the word, there is no intra-corpuscular glycolysis, but a complete oxidation of sugar.

TABLE 9

Summary of results

	CIRCULATING BLOOD				INCUBATED BLOOD			
	1 hour	2 hours	3 hours	Total	1 hour	2 hours	3 hours	Total
I. Showing percentage hourly loss of sugar from whole blood, serum or plasma and corpuscles and total percentage loss at end of three hours. Temp. 36°C.								
With heparine								
Blood	11.8	13.2	16.2	*37.9*	12.6	15.3	15.9	*38.8*
Plasma	6.5	11.6	15.7	30.4	6.4	11.5	11.5	30.3
Cells	22.2	16.9	26.4	52.3	24.6	24.3	16.3	52.2
With defibrinated blood								
Blood	13.3	16.3	20.7	*42.5*	13.2	11.7	15.5	*35.3*
Serum	8.3	12.9	20.4	39.6	10.3	14.4	14.0	34.6
Cells	26.2	21.2	22.1	55.0	27.3	27.5	25.3	60.6
II. Showing percentage hourly increase in lactic acid in whole blood, serum or plasma and corpuscles and total percentage increase in three hours								
With heparine								
Blood	Nil	Nil	3.2	*3.2*	Nil	0.7	10.5	*10.5*
Plasma	1.2	1.4	2.7	5.4	Nil	5.6	6.9	12.5
Cells	Nil	Nil	Nil	Nil	2.1	Nil	24.9	*12.4*
With defibrinated blood								
Blood	9.4	7.5	8.3	*27.4*	11.7	12.3	8.7	*32.8*
Serum	13.1	8.9	10.4	36.2	15.5	11.3	8.9	40.4
Cells	0.09	3.4	1.8	*5.5*	0.23	3.5	19.4	*14.1*

Table 6 shows the most marked alteration which is occasioned by defibrination, namely, a diminution in the amount of sugar oxidised with an increase in that glycolysed, the averaged figures being 70 and 30 per cent respectively. An examination of the percentage changes in the serum and corpuscles shows that this glycolysis takes place mainly in the serum and not in the cell.

It is clear however that the cell, although its own oxidative processes

have been somewhat diminished by defibrination is still the seat of the oxidative removal of sugar from the blood.

These results show that glycolysis does not take place in well oxygenated circulating blood to which heparine has been added, and that all sugar disappearing therefrom is oxidised by the corpuscles. This demonstrates for dog's blood a very facile passage of sugar through the corpuscular envelope. Further they show that defibrination impairs the efficiency of the oxidative mechanism within the cell.

2. *In incubated blood. Sugar utilisation:* A consideration of tables 7 and 8 shows that incubated blood to which heparine has been added loses sugar at a rate comparable to that in circulating blood, namely, for whole blood 12.23 mgm. per cent and for serum and the cells 6.4 and 5.8 mgm. respectively. When the blood is defibrinated results are practically similar 9.02 mgm. for the whole blood, 6.11 mgm. for the serum and 2.91 mgm. for the cells, the increased percentage loss in corpuscular sugar being due probably to damage of the corpuscles. The comparative loss of sugar under these two conditions is shown in table 9, the heparinised blood loses 38.8 per cent of its sugar the defibrinated blood 35.3 per cent. The loss of sugar from the corpuscles in heparinised blood is similar to that for circulating blood namely 54.2 per cent. Defibrination however causes a marked increase in the loss of corpuscular sugar.

Lactic acid: It has been shown (tables 5 and 6) that circulating blood to which heparine has been added suffers no increase in lactic acid while defibrinated blood does. Heparinised blood while standing in the incubator shows no increase in lactic acid during the first two hours. There is also no increase in the corpuscular lactic acid during the same period and in the experiment quoted the increase of 2.86 mgm. during the third hour is probably an error because such an increase in the third hour is ususual.

In incubated defibrinated blood the lactic acid changes are comparable to those obtaining in circulating defibrinated blood, there being however a greater increase in the corpuscular lactic acid of the incubated blood. The comparative percentage changes in lactic acid under the various experimental conditions mentioned are tabulated in table 9.

It is clear from such results that heparinised blood in circulation and well oxygenated maintains a constant lactic acid content. Incubation tends to permit of lactic acid formation in heparinised blood; defibrination definitely increases lactic acid formation, the increase being more marked in incubated than in circulating oxygenated blood. The cellular changes are definite: in heparinised blood in circulation the change in corpuscular lactic acid is *nil*, for the same blood in the incubator it is 12.4 per cent: in defibrinated blood in circulation it is 5.5 per cent and for the same blood in the incubator it is 14.1 per cent. The deleterious effect of defibrination with inadequate oxygenation is again manifest.

Glycolysis and oxidation: The results obtained with incubated blood (tables 7 and 8) are comparable to those obtained with circulating blood, namely, that heparinised blood oxidises most, if not all, its sugar, defibrinated blood oxidises approximately 50 per cent of its sugar. Table 7 indicates clearly what obtains in heparinised blood; of the blood sugar which disappears in the first two hours of the experiment 90 to 100 per cent is oxidised; the cell oxidises all of its sugar, and of the sugar removed from the serum 80 to 100 per cent is oxidised. Defibrination on the other hand certainly favours glycolysis in the serum and to a less extent in the cell.

It is apparent, from these results, that, in any estimation of changes in blood sugar, it would be essential to determine lactic acid changes, if a clear relation were to be established between sugar utilisation and oxygen consumption. It would also appear that where the CO_2 content of the blood is constant, any change in lactic acid would indirectly indicate whether or not oxygenation of the blood cell and oxidation within it were efficient.

SUMMARY

The distribution of sugar and lactic acid between serum or plasma and corpuscles in heparinised and defibrinated blood has been studied. It has been shown that defibrination produces a loss of corpuscular sugar and an increase in lactic acid both in the serum and in the corpuscles.

Variations in the relation between sugar glycolysed and oxidised have been demonstrated for heparinised and defibrinated blood, circulating and incubated, and it has been shown that the corpuscles of well oxygenated heparinised blood oxidises all the sugar which may disappear from the blood; that incubated heparinised blood suffers a little glycolysis; that of the sugar lost from defibrinated blood, well oxygenated or in the incubator, approximately 70 per cent is oxidised; that with defibrination the greater part of the glycolysis takes place in the serum or plasma the corpuscles retaining in a large measure their power to oxidise sugar.

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HYPERTROPHY OF THE GASTRO-INTESTINAL TRACT AND HIGH RESIDUE DIETS[1]

T. ADDIS

From the Department of Medicine of Stanford University School, San Francisco

Received for publication September 11, 1931

The following experiments were planned for the purpose of defining the conditions which are associated with an increase in the weight and length of the gastro-intestinal tract when the proportion of indigestible material in the food is increased. That there is a relation between the nature of the food and the size of the gastro-intestinal tract is rendered probable from a consideration of the length of the tract and the character of the food of various animal species (Magnan, 1911; Babak, 1903; and Fermi and Repetto, 1901); the fact that such a relation exists has been demonstrated by Wetzel (1928) and Kustner (1929) who found that the weight and length of the large intestine in rats was increased when vegetable food was given. In their experiments only the large intestine was altered, but it was hoped that by accentuating the difference between the proportion of residue in the control and experimental diets it might be possible to produce significant differences in other sections of the tract and thus obtain data which would facilitate an analysis of the various factors concerned in the process. Finally the rate of increase was determined for comparison with rates of increase observed under other experimental conditions in other organs.

At 26 days of age 88 ♂ albino rats were divided into two groups of littermates. The control group was given an adequate diet of the casein-starch-lard type which was relatively free from indigestible material. It formed a smooth compact paste. This will be called the "smooth diet." The experimental group was given a mixture which contained the same concentration of protein, vitamines and minerals but most of the starch and fat was replaced by finely ground dry alfalfa and agar-agar. It was a light homogeneous powder. Half of its weight consisted of cellulose-rich alfalfa (40 per cent) and of agar-agar (10 per cent). This will be called the "bulky diet." The percentage composition is given in table 1.

After 5 days on these foods 21 rats on the smooth diet and 19 on the bulky diet were killed and the degree of increase in the weight and length of the various sections of the gastro-intestinal tract was measured.

[1] This work was carried out with the help of a grant from the Josiah Macy, Jr. Foundation.

When the remaining 48 rats were killed after eating these foods for 44 days the bulky diet group did not have much more than half the body weight of the smooth diet group. The respective rates of growth are given in figure 1. Now 1 gram of the smooth diet was equal to nearly 5 calories, while 1 gram of the bulky diet had a caloric value of only 3.3 calories. But the supposition that the relatively defective growth on the bulky diet might be related to an inadequate caloric consumption is negatived by the observation, shown in the lower part of figure 1, that the actual caloric intake per 100 sq. cm. of body surface was greater on the bulky than on the smooth diet. It is true that no great degree of accuracy was attained in measuring the food intake with the bulky diet because the light powdery food was sometimes kicked out of the special food boxes by the rats. But this error touches only the degree of difference between the two groups, for any reasonable correction leaves the caloric intake greater on the bulky than on

TABLE 1

Percentage composition of "smooth" and "bulky" diets

SMOOTH DIET	per cent	BULKY DIET	per cent
Casein	16	Casein	16
Corn-starch (cooked and dried)	44	Corn-starch (cooked and dried)	11
Lard	17	Lard	0
Cod liver oil	10	Cod liver oil	10
Dry yeast	9	Dry yeast	9
Salt mixture (Osborne and Mendel)	4	Salt mixture (Osborne and Mendel)	4
		Dry alfalfa	40
		Agar agar	10

the smooth diet. Measurements made over the last 10 days showed that 1.2 grams of stool per 100 sq. cm. of body surface were excreted on the bulky diet as compared with 0.3 gram on the smooth diet. The appearance of the stools supported the assumption that the greater rate of excretion on the bulky diet was due in the main to cellulose and agar-agar. There was no indication of loss of fat. The presence of 10 per cent of cod liver oil and 9 per cent of yeast in the food seems to make a failure of vitamine absorption improbable. There does not seem then to be any very obvious reason for the observed difference in rate of growth.

In any case the difference in the final size of the 44 day groups makes it necessary to relate the gastro-intestinal measurements to some measure of body size in order to make them comparable. MacKay and MacKay (1927) have shown that the kidney weight per 100 sq. cm. of body surface is approximately constant for rats of all ages, and therefore for rats of all sizes, from 35 days old and onwards. Since the curve given by Donaldson (1924)

for the intestinal tract in relation to body weight is similar in form to the curve for the kidney in relation to body weight, it may be reasoned that the gastro-intestinal tract per unit of body surface should be comparable in our control and experimental series even though one group weighed nearly twice as much as the other. We have, accordingly, in each of our rats divided the gastro-intestinal weight by the body surface as calculated

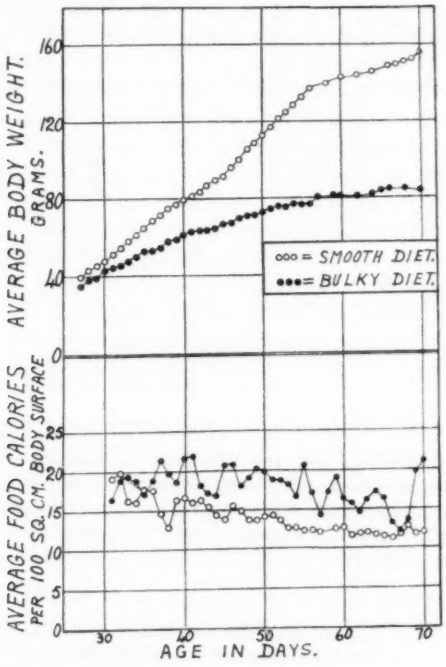

Fig. 1 Rate of growth shown in upper section of graph.
Food consumpton shown in lower section of graph.
Note that the food consumption is expressed in relation to body surface as calories per 100 sq. cm. of body surface and is therefore independent of the difference in body weight of the two groups.

from the body weight (body surface = 11.3 × body weight $^{\frac{2}{3}}$). This quotient multiplied by 100 gives the weight per 100 sq. cm. of body surface. The length of the gastro-intestinal tract was referred to the body length and is given as millimeters per 100 mm. of body length.

The individual results in the 44 day groups are given in figure 2 and figure 3 in terms of the percentage increases with respect to the control averages. The differences between the smooth and bulky diet groups are

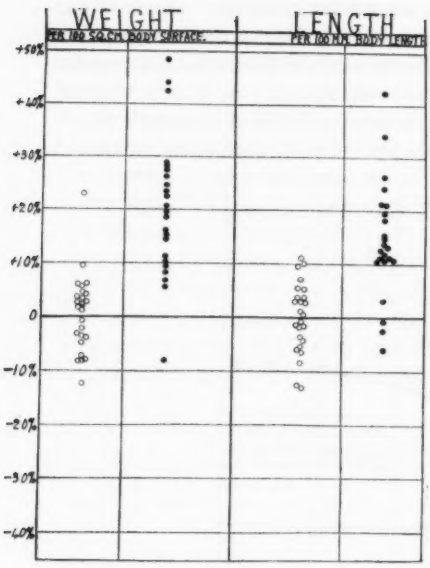

Fig. 2

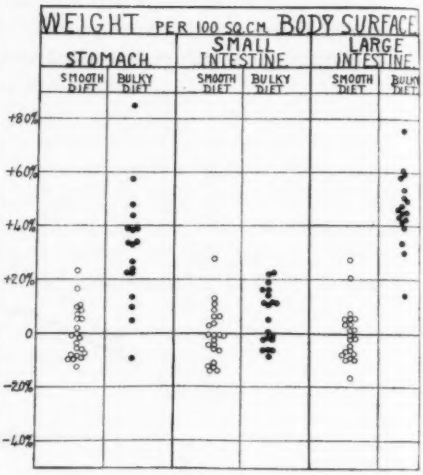

Fig. 3

Figs. 2 and 3. Circles represent the individual observations after 44 days on the smooth diet. Dots represent individual observations after 44 days on the bulky diet. Zero on the ordinate scale represents the average of the smooth diet group.

The weights and lengths of the gastro-intestinal tract of each rat are plotted as + or − per centage deviations from the smooth diet averages.

TABLE 2

The average percentage increase in weight and length on the bulky diet with reference to the average on the smooth diet

	TOTAL TRACT	STOMACH	SMALL INTESTINE	LARGE INTESTINE
After 5 days				
	per cent	*per cent*	*per cent*	*per cent*
Weight per 100 sq. cm. body surface	16	16	11	31
Length per 100 mm. body length	7	1	5	22
After 44 days				
Weight per 100 sq. cm. body surface	21	35	8	48
Length per 100 mm. body length	12	9	10	34

so great as to render any statistical evaluation unnecessary except in the case of the small intestine weights.

In table 2 the average percentage increases with respect to the control averages are given for both the 5 day and 44 day experimental groups. It will be noted that even in 5 days the average increases are considerable.

Discussion. The "bulky" diet taken by the experimental groups was the "smooth" diet of the controls diluted with an equal weight of finely ground alfalfa and agar-agar. But the physical differences in the diets as they were prepared—light and powdery in one case and heavy and compact in the other—would not be preserved after the foods had entered the gastro-intestinal tract. From the time when the two mixtures lay wet and acidified within the stomach until their residues reached the rectum, the difference between the two would be modified by digestion and absorption and by the addition and subtraction of water. But some conception of the stimulus to the changes in the stomach may be gained by comparing the weight of food taken by the control and experimental groups and their relative volumes as approximated from the volumes of acidified suspensions of each diet compacted by the centrifuge, while in relation to the changes in the large intestine the weight and volume of the stools provide a fairly satisfactory measure of the actually effective difference between the two diets.

The weight of food measured as taken during the last ten days per unit of body surface was 2.28 times greater in the experimental than in the control group, but since some of the bulky diet was lost by scattering it is better to estimate it as twice as great. The difference in the volumes occupied by equal weights of the smooth and bulky diets suspended in 0.1 N H Cl varied with the speed of centrifuging but at moderate rates, which might be thought of as approximating to the degree of compression which the musculature of the rat's stomach would produce, the volume of the bulky diet was twice that of the smooth diet per unit of weight. We may therefore think of the stomachs of the experimental group as working with twice the weight and four times the volume of the controls. The result in the 44 day groups was a 35 per cent increase in the weight of the stomach. There was only a 9 per cent increase in length but the length of the emptied stomach was obviously different from the length of the full stomach in the living rat, and so this measurement cannot be regarded as of much significance. In the 5 day groups, assuming the same differences, the result was a 16 per cent increase in stomach weight and a 1 per cent increase in length.

The weight of the stools per unit of body surface was four times greater in the experimental than in the control group, and per unit of weight the volume was 1.4 times greater. We may therefore consider that the rectum and, in a modified degree, the large intestine of the rats on the

bulky diet had to forward four times the weight and 5.6 times the volume of stools. The result in the 44 day groups was a 48 per cent increase in weight of the large intestine and a 34 per cent increase in length. In the 5 day groups there was a 31 per cent increase in weight and a 22 per cent increase in length. In these groups the cecum was measured separately and a 51 per cent increase in weight and a 25 per cent increase in length was observed.

We have no data from which to obtain even an approximate idea of the quantitative difference between the two diets in the small intestine. But

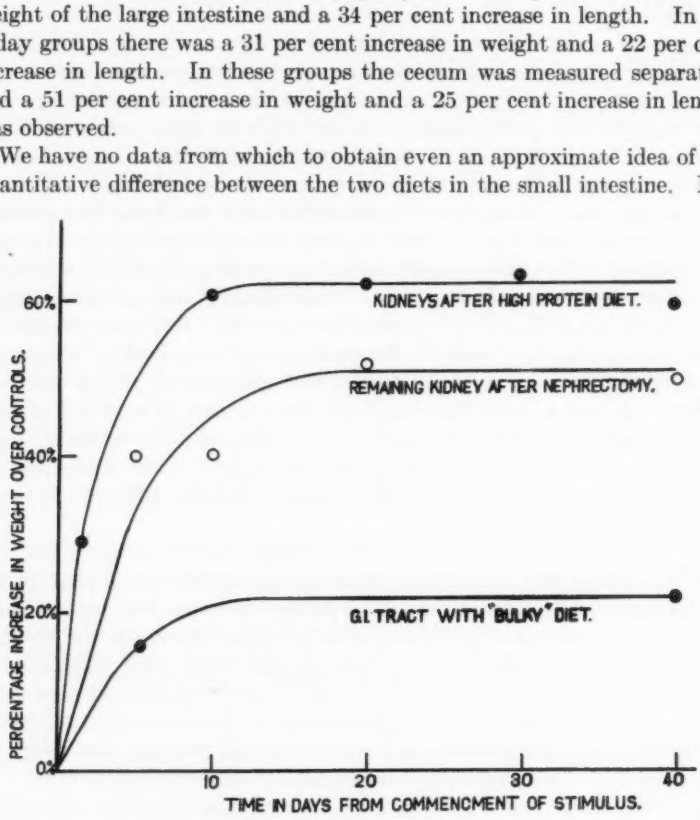

Fig. 4

inspection showed that in both the control and the experimental animals the contents were fluid or semi-fluid. After 44 days there was an 8 per cent increase in weight and a 10 per cent increase in length in the experimental group and after 5 days the increases were 11 per cent and 5 per cent respectively.

The measurements given do not warrant an attempt to describe the results in mechanical terms since we do not know the effect of the difference in the diets on the work done by the various sections of the gastro-intestinal tract. But it is clear that the increase in weight and length of the stomach and large intestine where the food was in a semi-solid or solid state is much

greater than in the small intestine where the work required in moving the material is lessened because there the particles float in fluid. The observed changes are interpreted as an adaptation to increased work,—a work hypertrophy.

In figure 4 the rate of increase in the weight of the gastro-intestinal tract in the rats on the bulky as compared with the rats on the smooth food is charted for comparison with two rates of increase in kidney weight which are regarded as also due to an increase in work, the first because of an increase in protein consumption, the second because of the removal of one kidney and a consequent doubling of the work done by the remaining kidney.

In these three experiments the age and sex of the animals was the same and in each case the stimulus to the organ hypertrophy was derived from the ingestion of food. The characteristic of all three is an approach to a maximum at about 15 days after the imposition of the excess load. We have data which suggest another form of the curve of rate of increase where the stimulus to hypertrophy is not of such a nature as to be thought of in terms of "work."

CONCLUSIONS

1. The effect of a high residue diet on the weight and length of various sections of the gastro-intestinal tract has been determined.

2. The results are interpreted as an example of work hypertrophy. The curve of rate of increase is similar in form to curves of the rate of increase in kidney weight under the stimulus of increased work.

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THE SEDIMENTATION OF ERYTHROCYTES SUSPENDED IN LYMPH

JOHN RUSSELL AND T. E. BOYD

From the Department of Physiology and Pharmacology, Loyola University School of Medicine

Received for publication September 14, 1931

In the literature of recent years on blood sedimentation, we have seen no record of studies on artificial suspensions of erythrocytes in body fluids other than plasma. The experiments here reported deal mainly with such suspensions, in lymph from the thoracic duct of the dog. It is well known that in pregnancy, and in certain diseases, the sedimentation of whole blood is much more rapid than that of blood from healthy non-pregnant subjects. It has been shown that this phenomenon depends on some property of the plasma rather than of the corpuscles, though it is influenced by the volume relations between the two. Using as suspension media specimens of lymph collected from a series of animals, we wished to determine whether or not differences in the rate of sedimentation would appear parallel to those found with corresponding samples of plasma. We were interested also in comparing, as suspension media, lymph and plasma from the same animal. As the two fluids differ mainly in the relatively low protein content of the former, such a comparison might help in evaluating the influence of the plasma proteins on sedimentation. It has been pointed out, notably by Gram (1921), that rapid sedimentation in whole blood is associated with a high concentration of plasma globulins, and particularly of fibrinogen. In artificial suspensions, the volume ratio of corpuscles to liquid can be kept constant for all the preparations to be compared. If fibrinogen is the most important remaining factor, corpuscles suspended in lymph should settle more rapidly than in physiological saline solution, less rapidly than in plasma.

We have made such comparisons on a series of nine dogs. In addition, we have administered peptone intravenously to eight of them, to determine whether or not it would affect the properties of lymph as a suspension medium. It is known that peptone, like other lymphagogues of Heidenhain's first class, will increase the protein concentration of lymph. The effect on the lymph fibrinogen content is not entirely clear from the literature. Peterson, Jaffe, Levinson, and Hughes (1923) report that the injection of Witte's peptone, in amounts of 50 to 100 mgm. per kilo, rendered

the lymph temporarily incoagulable. This, like the incoagulability of blood under similar conditions, was probably due rather to the presence of an anticoagulant than to the absence of fibrinogen. It is noticeable in their protocols that the first sample of lymph to coagulate, after a peptone injection, usually gave a higher yield of fibrin than did normal lymph from the same dog.

PROCEDURE. Dogs of medium or large size were used. Preliminary tests were made on each animal, and they were so selected that slow, rapid, and medium sedimentation rates for whole blood are represented in the series. No food was given for 24 hours before the experiment. As an anesthetic, sodium barbital, 0.35 gram per kilo body weight, was given by stomach tube. It was sometimes necessary also to administer a little ether. To obtain corpuscles and plasma, we drew from the femoral vein 45 cc. of blood, diluting it in the syringe with 5 cc. of 4.4 per cent potassium oxalate solution (Greisheimer, Ryan and Johnson, 1929). This was centrifuged, the plasma removed and the corpuscles washed twice with 0.9 per cent NaCl solution.

The thoracic duct was cannulated, and lymph collected in sedimentation tubes of the type described by Cutler (1926). One-half cubic centimeter of a 4.4 per cent solution of potassium oxalate was put into each tube, and lymph allowed to fill it to the 5 cc. mark. Ordinarily, we collected two samples of "normal" lymph, then administered peptone and collected additional samples at intervals afterward.

Fibrin determinations were made by the method of Wu (1921), 1 cc. portions of plasma and 2 cc. of lymph being used. Special care was taken in collecting the fibrin from clotted lymph, on account of the fractional coagulation described by Lusky (1910). The fibrin was removed on a small stirring rod every half hour until no more could be obtained. The lymph was again examined after 24 hours. If no coagulum was then present it was assumed that all of the fibrin had been removed.

A series of sedimentation tubes was set up, each tube containing 2 cc. of corpuscles and 3 cc. of suspension fluid. One tube contained plasma, one 0.9 per cent NaCl, and the remaining tubes the various samples of "normal" and "peptone" lymph. "Normal" lymph means here simply that it was collected previous to the use of a lymphagogue, and implies nothing as to the physical condition of the animal. The tubes were allowed to stand at room temperature, and readings were made at 1, 3, and 24 hours. For the sake of brevity only the 1-hour readings are given below. In the peptone experiments we also measured the sedimentation velocity of whole blood, one sample drawn before and a second drawn one to one and one-half hours after the administration of peptone.

RESULTS. 1. *Sedimentation and fibrin content in plasma and "normal" lymph.* Table 1 shows sedimentation readings for the nine animals, in

plasma, normal lymph, and 0.9 per cent saline solution. While the 1-hour readings for plasma range from 14 mm. down to 2 mm., those for lymph are all 2 to 2.5 mm. Lymph suspensions showed a slightly more rapid average sedimentation rate than saline suspensions, but the difference is small, and in six experiments out of nine the readings were identical. The three-hour readings, not shown in the table, were also approximately the same for the two fluids.

The fibrin yield from plasma ranged from 0.528 gram down to 0.272 gram per 100 cc. The extremes for lymph were 0.186 and 0.133, indicating a smaller degree of variability. It is evident that the fibrinogen content of plasma may be high without a corresponding rise of its concentration in lymph.

TABLE 1

Sedimentation and fibrin estimations on plasma and "normal" lymph

The dogs used in experiments 2, 4, and 9 were healthy males; 5, 7, and 8 pregnant females, and the remaining three males with "snuffles."

EXPERIMENT	SEDIMENTATION, 1 HOUR			FIBRIN	
	Plasma	Lymph	0.9 per cent NaCl	Plasma	Lymph
	mm.	*mm.*	*mm.*	*gram per 100 cc.*	*gram per 100 cc.*
1	13.0	2.5	1.5	0.362	0.160
2	3.5	2.0	2.0	0.291	0.133
3	14.0	2.0	2.0	0.386	0.148
4	3.0	2.0	2.0	0.272	0.152
5	14.0	2.0	2.0	0.380	0.158
6	14.0	2.0	1.5	0.528	0.186
7	9.5	2.0	1.5	0.370	0.134
8	12.5	2.0	2.0	0.470	0.145
9	2.0	2.0	2.0	0.312	0.156

2. *The effect of peptone.* Peptone was administered in ten per cent solution, by way of the femoral vein. Four experiments were carried out with Witte's peptone, 100 to 500 mgm. per kilo, and four with Armour's, 100 to 650 mgm. The sedimentation rate and fibrin content of lymph were in all instances increased. In six of the eight experiments the effect appeared in the first sample of lymph collected after the injection. The increase was invariably found in samples drawn after an interval of 15 minutes to 1 hour. We did not continue all experiments until the "normal" values were restored. Two of the eight animals showed a return to normal or below within one and one-half hours. In the remaining six the final lymph sample (collected one to one and one-half hours after the peptone was administered) still showed high fibrin and sedimentation figures. The following table is typical.

In no instance did the peptone lymph fail to clot after dilution and addition of $CaCl_2$. The difference between our observations and those of Petersen, Jaffe, Levinson, and Hughes may perhaps depend on differences in the rate of peptone injections. Our injections, particularly of Witte's peptone, were made slowly.

It may be noted that peptone increased the fibrin in plasma, and the sedimentation velocity in plasma-corpuscle suspensions. The sedimentation rate for whole blood was in our experiments somewhat reduced. This was probably due to the relative increase in corpuscle volume in whole blood, a change which more than offset the effect of the increased fibrinogen.

TABLE 2

Effect of intravenous peptone injection on fibrin yield from lymph and on sedimentation in lymph-corpuscle suspensions

Experiment March 12, 1930. Male dog, snuffles, 9.8 kilos. Witte's peptone, 100 mgm. per kilo.

SUSPENSION MEDIUM	SEDIMENTATION, 1 HOUR	FIBRIN
	mm.	*gram per 100 cc.*
0.9 per cent NaCl	1.5	
Normal plasma	13.0	0.362
Normal lymph (1)	2.5	0.159
Normal lymph (2)	2.5	0.161
Lymph collected after peptone injection:		
5 minutes	3.5	0.190
60 minutes	3.0	0.182
90 minutes	3.5	0.203
Plasma, drawn 90 minutes after peptone injection	13.5	0.382

DISCUSSION. Most of our observations are in accord with the prevalent belief as to the importance of fibrinogen in determining sedimentation rate. Thus in plasma taken from the diseased or pregnant animals of the series, sedimentation and fibrinogen figures are high, as compared to those obtained with healthy non-pregnant dogs. The low and relatively uniform sedimentation rates found in lymph (except the peptone samples) were associated with fibrin yields also low and uniform, as compared to those from plasma. Peptone lymph as compared to normal lymph shows a more rapid sedimentation rate and an increase in fibrinogen.

The effect of peptone on sedimentation rate, however, seems disproportionately great compared to its effect on fibrin content. It is possible that the available methods of analysis, which require complete coagulation, do not measure all of the fibrinogen in peptone lymph. The fact that normal lymph and 0.9 per cent saline solution behave so nearly alike as suspension media is also difficult to reconcile with the supposed influence of fibrino-

gen on sedimentation. It may be assumed, perhaps, that there are other substances, present in lymph but absent from saline, which tend to retard sedimentation. Gram (1921) found that corpuscles in a solution of pure fibrinogen settled somewhat more rapidly than in saline solution. The difference he noted was not great, however, and the concentration of fibrinogen used (0.19 per cent) was higher than in any of our samples of "normal" lymph.

SUMMARY

1. Corpuscles suspended in thoracic duct lymph from the dog settle more slowly than in plasma. The sedimentation rate in "normal" lymph suspensions is approximately the same as in 0.9 per cent NaCl solution.

2. In a series of dogs including diseased and healthy males and pregnant females, the plasma fibrinogen and sedimentation figures were found to show parallel variations similar to those well known to occur in human subjects. Variations in lymph fibrinogen content, however, were relatively slight, and the sedimentation velocity in lymph-corpuscle suspensions were correspondingly uniform.

3. The intravenous administration of peptone is followed by an increase in the fibrinogen content of thoracic duct lymph, and a more rapid sedimentation rate in lymph-corpuscle suspensions.

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THE RATE OF DISAPPEARANCE OF GLYCOGEN DURING CONTRACTION OF THE PERFUSED HEART OF THE RABBIT

WALTER E. MACPHERSON,[1] HIRAM E. ESSEX AND FRANK C. MANN

From the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minnesota

Received for publication August 26, 1931

Experiments reported by Witting, Markowitz and Mann (1930) demonstrated that the hearts of rabbits in the course of perfusion with glucose-free Ringer-Locke's solution, showed a marked fall in glycogen, and that the hearts survived about four hours. They also observed that if such hearts were perfused with a solution containing glucose, they remained active for as long as eight or nine hours. The experiments reported herewith were undertaken for the purpose of determining the approximate rate of disappearance of glycogen during contraction of the heart of the rabbit while it was being perfused with oxygenated Ringer-Locke's solution, with and without glucose.

METHOD OF EXPERIMENTS. The type of research made it necessary to develop a method of securing specimens repeatedly from the same heart for the estimation of glycogen. This was accomplished by removing numerous sections of the muscle of the heart at specified intervals and analyzing them for glycogen content. After a considerable number of hearts had been perfused and sectioned during the perfusion, it was demonstrated that such sections could be removed without definitely interfering with the contractions of the remaining portion so long as the auricles remained intact. Transverse sections weighing approximately 1 gram each were removed with a sharp razor at hourly intervals, beginning at the apex and ascending toward the base. The last part of the heart to be removed was the auricles.

After the rabbit had been etherized, the abdomen was opened and the abdominal aorta and vena cava were severed, thereby allowing the animal to bleed to death. The thorax was then opened, the heart carefully removed and washed quickly with glucose-free Ringer-Locke's solution until it was free from blood. It was then ready to be perfused after the manner of Locke and Rosenheim with the perfusing fluid at a temperature of approximately 37°C. Having satisfied ourselves that sections could be

[1] Special student.

removed from the perfused heart without definitely interfering with the activity of the remaining portion, the following experiments were performed.

As controls, eight rabbits' hearts were analyzed for glycogen content by the method of Pflüger, with slight modifications. The controls were prepared as for perfusion by washing them with glucose-free Ringer-Locke's solution until they were bloodless; they were weighed quickly and placed, whole or in part, into an equal quantity of saturated potassium hydroxide solution at the temperature of boiling water. After hydrolysis of the glycogen, the sugar was estimated by the Folin method. The glycogen content was expressed as glucose. Three hearts were placed in the potassium hydroxide solution without being cut into sections in order to learn whether the sectioning greatly changed the amount of glycogen present. Five of the controls were cut into transverse sections weighing approximately 1 gram each, and these sections were properly analyzed for their glycogen content. The auricles were separated from the associated vessels and were prepared, to ascertain the relative amounts of glycogen present as compared to the ventricles. Because the glycogen disappears rather quickly from injured muscles, the injury from sectioning the hearts was brought to a minimum by removing all sections with a sharp razor. All of the perfused hearts were sectioned in the same manner as were the controls.

The perfused hearts were divided into two main groups: those perfused with Ringer-Locke's solution containing glucose, and those perfused with the same solution without glucose.

Since the glycogen content of the various hearts seemed to differ considerably, it seemed advisable to remove control sections from the hearts that were to be perfused. In four of the six hearts which were perfused with solutions containing glucose, two or three sections were removed from the ventricles immediately after the hearts had been washed and these were prepared for determinations of glycogen. This gave us a control for each heart. The hearts were then perfused. They remained apparently normally active, and were sectioned during the time of perfusion in the customary manner.

Ventricular sections were removed for controls from the heart that were perfused with glucose-free solution. They were then perfused in the customary manner, and sections were removed at hourly intervals.

It should be mentioned that the perfused hearts were never allowed to stop contracting; definite activity was visible at all times.

Results. A control series of eight rabbits' hearts disclosed that the average glycogen content was 0.483 per cent, with a minimum of 0.334 per cent and a maximum of 0.64 per cent. The comparative amounts of glycogen varied at different parts of the heart, being slightly highest in the auricles, next highest at the apices, and less in amount toward the bases of the ventricles. Even though the total quantity of glycogen varied con-

siderably in different hearts, the comparative amounts at different levels of each heart were consistently similar. The amount of glycogen in the sectioned hearts was apparently not definitely lowered by the procedure, and the glycogen content did not fall below the level of that in the whole hearts.

Owing to the variations of glycogen percentage in different hearts, sections were removed from the ventricles of most of the perfused hearts to be used as approximate controls for the individual hearts. In all the perfused hearts the glycogen invariably fell below the level of the individual control as well as below the level of the average control.

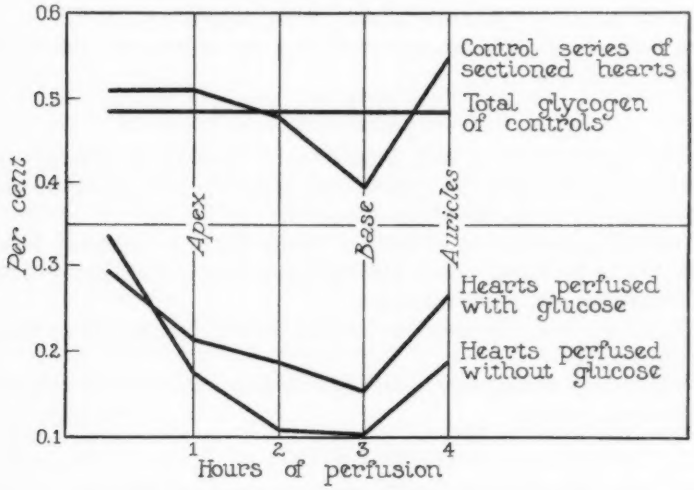

Fig. 1. Average glycogen content of entire series of hearts studied. The initial value shown in the first, third and fourth graphs was obtained from a portion of the ventricle.

In the hearts perfused with Ringer-Locke's solution containing glucose, the glycogen content fell rather gradually to reach the approximate minimum of 0.152 per cent within a period of three hours. From the controls it was noted that the auricles, the portion of the heart which is most irritable and which will beat longest, contain the most glycogen and even after four hours of activity contain a fairly high percentage.

In the hearts perfused without glucose in the perfusing fluid, a more rapid drop in glycogen content occurred during the first hour, almost reaching a minimum in the ventricles at the end of the second hour. The glycogen content of these hearts dropped much lower at a more rapid rate than in those hearts perfused with glucose. Here again, the glycogen content of the auricles was still fairly high at the end of the period of perfusion.

In comparing the perfused hearts with the controls, it would seem that the greatest drop in glycogen content of the entire heart occurs during the first hour and is very gradual after that. It should be noted that this rapid decrease in glycogen content, particularly during the first hour, may be owing to the changed environment of the cardiac muscle and not to the metabolism of glycogen. The average values obtained are shown in figure 1.

SUMMARY. Rabbits' hearts were analyzed for glycogen content. The hearts were cut into sections and the glycogen ascertained for various levels. One series was analyzed for control purposes, another series was perfused with Ringer-Locke's solution containing glucose, and third series was perfused with the same type of solution but free of glucose.

The methods of perfusing and sectioning the hearts, and the results obtained, are given.

CONCLUSIONS

1. The ventricles of perfused rabbits' hearts continue to contract after large amounts of ventricular muscle are removed. The activity of such hearts apparently is not definitely shortened.

2. The glycogen content of rabbits' hearts varies at different levels; it is highest in the auricles, next highest at the apex, and gradually diminishes toward the base of the ventricles.

3. More glycogen disappears in the first hour of perfusion than at any succeeding time.

4. The glycogen disappears faster when the hearts are perfused with solution free from glucose.

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SOME CHEMICAL EFFECTS FROM CONSTANT INTRAVENOUS EPINEPHRINE INJECTION IN DOGS

PAUL C. SAMSON[1] AND H. R. D. JACOBS[2]

With the technical assistance of HULDA ROHDE

From the Department of Medicine, Rush Medical College of the University of Chicago, Chicago, Ill.

Received for publication September 24, 1931

The many effects of epinephrine given to animals in single or repeated doses have been studied by numerous observers, and most of the phenomena appearing in this mode of attack have become common knowledge (1).

It is the purpose of this paper to present data gathered from observations on dogs to whom epinephrine was given continuously over relatively long periods of time, in contrast to the intermittent, large dose method.

EXPERIMENTAL METHODS. By means of an apparatus described by one of us (2), epinephrine was injected at a constant rate into the jugular vein of dogs. The animals used were normal non-pregnant females of average size. They had the freedom of their cages during the experiment and were not anesthetized or hampered in any way except by the collar and chain of the apparatus.

After a period of twenty-four hours without food, the dogs were attached to the apparatus and, under local anesthesia the intravenous cannula was inserted. Following the operation, physiologic saline solution was injected for a day or two at a constant rate, and during this time "normal" data on blood and urine were secured. Epinephrine diluted in physiologic saline solution was then injected.

Blood samples were taken from the femoral artery and saphenous vein and were analyzed for sugar content both according to Folin-Wu (copper method) (3) and Folin and Malmros (ferricyanide method) (4). Blood for the Folin-Wu determinations was collected in dry potassium oxalate tubes. The arterial blood for the Folin and Malmros method was taken from the oxalate tube, and the venous blood directly from the vein by means of a needle puncture. Because of the large number of blood samples taken it was decided early in the work to use the Folin and Malmros micro

[1] James B. Herrick Fellow in Medicine, on the Frank Billings Foundation, Rush Medical College.

[2] Ernest E. Irons Fellow in Medicine, on the Frank Billings Foundation, Rush Medical College. Under the supervision of Dr. George F. Dick.

method of blood sugar determination. However parallel determinations on the same blood using both the Folin iron and Folin-Wu methods showed divergent results in certain phases of the glycemic curves. In such cases duplicate and blank determinations ruled out the probability of error in method. The description of results and the comments will be based on the Folin-Wu figures only.

The total urinary excretion was measured in twenty-four hour periods. The bladder urine was obtained by catheterization at the end of each period. The urine was analyzed for sugar according to the method of Folin and Berglund (5); for total nitrogen by the Kjeldahl method.

Epinephrine-Mulford ("Adrin 1–1000") was used. Dilutions were made in physiologic saline solution. The rate of administration was approximately 0.001 to 0.002 mgm. per kgm. per minute (0.060 to 0.120 mgm. per kgm. per hour). These doses were chosen as being well within the maximum physiologic limit of secretion, considered by Kodama (6), and Cannon and Rapport (7) to be about 0.005 mgm. per kgm. per minute. Because of the tendency of epinephrine to deteriorate, fresh dilutions were made every twenty-four hours. The original weight of the animal was used as a basis for dosage.

After starting the experiment the dogs were given no food, but were allowed water as desired. Care was taken not to excite them during any part of the work in order to obviate the possibility of a hyperglycemia from this cause. If for any reason it became difficult to obtain blood samples, the attempt was postponed until later.

EXPERIMENTAL DATA. Four experiments were carried out, which lasted for from four to thirteen days. One representative chart and two protocols are included. Similar data were obtained from the other dogs. Unless otherwise specified the fluid administration in each experiment was uninterrupted from beginning to end, but, for convenience in expressing epinephrine dosage, urine collection, etc., the time is divided into "periods" of approximately twenty-four hours each.

RESULTS. *1. Effects on blood sugar.* The salient features of the glycemic curves are illustrated by the graph. In each experiment the epinephrine hyperglycemia reached a maximum fairly rapidly (3–5 hours). This maximum did not persist, and there was a gradual fall to normal blood sugar values over a period of about twenty-four hours. With continued epinephrine injection the blood sugar concentration stayed within normal limits (80 to 100 mgm. per cent), and even when the rate of administration was doubled at the end of twenty-four or forty-eight hours, a hyperglycemic phase did not follow, in fact, the effect of the added epinephrine was scarcely perceptible. However, if epinephrine was suddenly stopped while the blood sugar concentration was within normal limits, there was an abrupt drop to a hypoglycemic level for several hours and then a return to

Protocol 1 (see chart). Dog 106. Weight 13.6 kgm. Physiologic saline solution for 21 hours before beginning of epinephrine. Average hourly fluid injected: 24.7 cc. Blood sugar at end of "normal" period, 93 mgm. per cent.

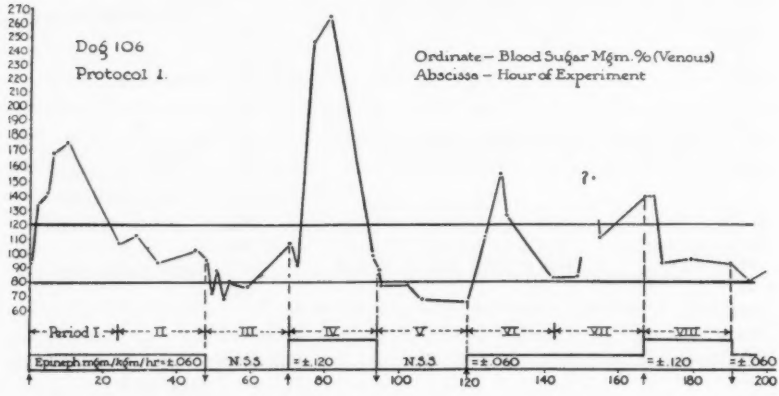
HOUR OF EXPERIMENT	PERIOD	FLUID FOR PERIOD	EPINEPHRINE	URINE FOR PERIOD	URINE SUGAR	URINE N	BLOOD SUGAR (FOLIN-WU)		REMARKS
							A.	V.	
		cc.	mgm./kgm./hr.	cc.	gm.	gm.	mgm. per cent	mgm. per cent	
0								93	Epinephrine begun
2								133	
4½		—	—	—	—	—		141	
6½								168	
10								174	
24	I (end of)	565	0.062	1,300	7.73	7.84		107	
29								112	
34								93	
44								102	
48	II	600	0.064	1,460	0.18	4.4		94	Epinephrine stopped
49¼								71	
50½								89	
52¼		—	—	—	—	—		69	Dog weak
54½								80	
59½								78	
71	III (23 hr.)	550	0.000	950	0.14	5.3		108	Epinephrine begun
73								91	Dose doubled
77								241	Dog stronger
81		—	—	—	—	—	—	265	
93¾								99	
94¾	IV	600	0.128	1,950	3.03	5.9		89	Epinephrine stopped
95								78	
95¾							98		
96¾		—	—	—	—	—	81		
97¾							84		
98¾							78		Dog weak
102¾								79	
106¾							86	69	
118¾	V	580	0.000	1,010	0.20	5.7	74	68	Epinephrine begun
123½							114	111	
127½							160	154	
129½							133	127	
142½	VI	590	0.064	1,910	0.26	4.7	100	82	
148½							98	83	
152½								151?	
154½							111	111	
167	VII	588	0.062	1,440	0.14	4.2	142	140	Epinephrine increased
169							142	140	
171½							94	92	
178							108	96	
190							109	94	

Protocol 1—Concluded

HOUR OF EXPERIMENT	PERIOD	FLUID FOR PERIOD	EPINEPHRINE	URINE FOR PERIOD	URINE SUGAR	URINE N	BLOOD SUGAR (FOLIN-WU)		REMARK
							A.	V.	
		cc.	*mgm./ kgm./ hr.*	*cc.*	*gm.*	*gm.*	*mgm. per cent*	*mgm. per cent*	
191½	VIII	540	0.115	1,240	0.14	3.3			Epinephrine de-
195½							89	80	creased. Food be- gun (meat)

This experiment was continued for another 100 hours, epinephrine being injected at a rate of 0.064 mgm./kgm./hour. The blood sugar values remained within normal limits during this time. Three times after meat was given there was a negative arterio-venous difference in blood sugar values.

Including the fore-period, the injection lasted continuously for approximately 308 hours. A total of 245.78 mgm. of epinephrine was given during this time.



normal. If epinephrine injection was then begun again there followed another hyperglycemic phase with recovery, and when stopped, a hypoglycemic phase. When the same rate of epinephrine administration was used *following* a rest period the second hyperglycemia was not as marked or as prolonged as the first. In protocol 1, period IV, when the rate of administration was doubled following a rest period, the blood sugar values were about one and one-half times those of the original hyperglycemia.

The hypoglycemia following the cessation of epinephrine administration could be demonstrated in fifteen minutes (protocol 1, period V). In another experiment the blood sugar values dropped from 98 to 61 mgm. per cent in an hour. It is interesting to note that in two of the dogs (proto-

cols 1 and 3) a period of weakness and lassitude paralleled the period of hypoglycemia. Return of strength was coincident with the return of blood sugar values to normal.

In protocol 1 the arterial values according to the Folin-Wu method were consistently higher than the venous, and the arterio-venous difference, which was small, was not increased during the hyperglycemic phase. This observation was further checked from time to time in the other experiments by the analysis of occasional venous samples. Such findings agree with the work of Cori (8) and others (9), on the arterio-venous blood sugar difference during epinephrine injection.

The Folin iron figures are included in the results for the reason that they exhibit several fairly constant features.

1. Only a transient hypoglycemia was obtained by this method.
2. Several times when this method gave normal blood sugar values, the Folin-Wu method gave very low values, more or less substantiated by symptoms of hypoglycemia in the animal.
3. Whereas with the first hyperglycemia the results with both methods checked fairly closely, with the second rise (protocol 3, period IV) the Folin iron values were considerably higher, both methods, however, giving normal values in about the same length of time.
4. This method showed a wider variation in the arterio-venous blood sugar differences than the Folin-Wu method. The venous blood sugar concentration was occasionally much higher than the arterial.

In a test experiment an insulin hypoglycemia in a dog was followed by both methods. There was agreement throughout the hyperglycemia phase, but for three hours after the Folin-Wu method showed a definite hypoglycemia, the Folin iron method gave normal values. Substitution of dilute lactic acid solutions for blood filtrates gave negative results by both methods.

2. *Effect on urinary output.* The effect of epinephrine on urinary output was to increase the excretion of urine for a twenty-four hour period in every case. This was true whether or not there was a definite glycosuria. The effect is best illustrated in protocol 3. "Normal" urinary output in twenty-four hours with a constant rate of injection of physiologic saline solution was about 400 cc. With epinephrine administration (the total fluid injected being the same as before), the volume rose to over 1400 cc. for four twenty-four hour periods. In only the first of these periods was there an increase in urine sugar. The same effect is shown to a lesser degree by the other dogs. Such findings are interesting in view of the contradictory reports in the literature as to whether epinephrine injection causes diuresis (10), or a suppression of urine (11).

3. *Effect on total urinary nitrogen excretion.* Continuous epinephrine administration had no definite effect on total urinary nitrogen excretion,

Protocol 3. Dog 108. Weight 10 kgm. Physiologic saline solution injected 44 hours before beginning epinephrine. Average hourly fluid injected: 27 cc.

HOUR OF EXPERIMENT	PERIOD	FLUID FOR PERIOD	EPINEPHRINE	URINE FOR PERIOD	URINE SUGAR	URINE N	BLOOD SUGAR (F-M) A.	BLOOD SUGAR (F-M) V.	BLOOD SUGAR (F-W) A.	BLOOD SUGAR (F-W) V.	REMARKS
		cc.	*mgm./kgm./hr.*	*cc.*	*grams*	*grams*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	
0—operation							127	156	140	140	Physiologic saline begun
4							109	120	97	97	Catheterized
25½							113		103		
44	end fore-period	1,289	0.000	450 (24 hr.)	0.28	3.53	82	95	80	80	Epinephrine begun
45							188	162	166		
46½							234	192	254		
48		—	—	—	—	—	234	238	244		
54							176	156	162		
68	I (end)	657	0.062	1,450	0.67	2.32	156	144	137		Epinephrine ½ above rate for 3 hr.
71							100	125	100		Epinephrine full rate
75							126	129	123		
92	II a— 3 hr.	40	0.030								
	b—21 hr.	570	0.061	1,410	0.22	2.00	140	133	131		Epinephrine stopped 1 hr.
93							74	86	74		Epinephrine full rate
98							87	111	90		
115½							91	94	91		
117							108	102	103		
119	III (27 hr.)	711	0.061	1,440 (25 hr).	0.22	1.73	109	104	103		Epinephrine stopped
119½							79	94	84		Dog appears weak
120							76	90	88		
121							95	90	99		

123	—	—	—	—	—	—	85	90	90		
125							93	85	88		
137							91	80	69	67	
140	IV (21 hr.)	556	0.000	355 (23 hr.)	0.12	1.77	72	72	69	67	Epinephrine begun
141½							86		100		Dog appears stronger
144¾							228	168	142		
147							206	252	164		
160							125	117	99	95	***
164	V	651	0.061	1,470	0.19	1.70	97	131	90	99	All injection stopped
165							82	113	73		
166							103	84	78		
168	—	—	—	—	—	—	123	121	63		
170½							70	82	57		
172							93	95	57		
184				Incomplete							Dog died

*** Very peculiar type of paralysis has developed, a combination of atonic and spastic paraplegia. Dog remains quiet, legs outspread. When an attempt is made to rise, dog whirls and falls. Many generalized muscular twitchings. Claws get caught in cage floor grating (probable flexor spasm). While lying, animal is limp, but it presents extreme muscular resistance on any attempt to move it. Marked salivation. Ante-mortem blood calcium normal (9.5 mgm. per cent). Death evidently due to a respiratory paralysis. No Negri bodies found on examination. Total time of injection, 164 hours. Total amount of epinephrine given, 59.2 mgm. Weight at death, 9 kgm.

measured by twenty-four hour periods, and probably therefore had no effect on protein metabolism. Although there was a gradual fall in total nitrogen excretion during the course of any one experiment, the daily variation was small whether physiologic saline or epinephrine was injected. This variation was generally less than 0.8 gram per day. A comparison of average values for total nitrogen excretion on the days when epinephrine was injected and on the days when no epinephrine was injected, showed no definite tendency in either direction. A terminal rise in total nitrogen excretion occurred only when the dogs were being fed.

Similar conclusions regarding total nitrogen excretion have been reached by Paton (12), and Underhill and Closson (13). Watkins and Smith (14) however, by finding an increase in the blood urea after epinephrine administration and no evidence of urea retention, believed that the effect of epinephrine was to increase protein catabolism.

4. *Effect on glycosuria.* The effect of epinephrine on glycosuria roughly paralleled that on glycemia. The initial glycosuria was variable. In protocol 1, epinephrine injection led to an excretion of 7.73 grams in twenty-four hours. In experiments 2, 3, and 4, the initial excretions were 1.27, 0.67, and 1.20 grams respectively for the first twenty-four hour period.

In each dog the urine was sugar free at the end of twenty-four hours of epinephrine injection, and there was no further glycosuria even though the rate of administration was doubled. This was further emphasized in a preliminary experiment in which, after an initial glycosuria, the urine became sugar free. There was no further glycosuria in spite of the fact that the original dose (0.001 mgm/kgm/minute) was increased each day for four successive days. The final dose (0.005 mgm. per kgm. per minute) was rapidly fatal.

There was generally a slight glycosuria during the period of a second epinephrine administration following a rest interval, but it did not equal the first in degree.

Such results are in agreement with those of Underhill and Closson (13), Herter and Richards (15), and Pollak (16), who gave repeated injections of large amounts of epinephrine. They found that the initial glycosuria rapidly decreased until the urine became sugar free, in spite of further injections. If, after a day or two without epinephrine, injections were again started, the glycosuria reappeared, but smaller in amount than the original.

COMMENT. The cause for the differences in blood sugar values as determined by the Folin-Wu and Folin and Malmros methods is not evident. It is conceivable that the higher values by the Folin iron method are due to a non-sugar substance, and any of the fragments of hexose degradation, as well as lactic acid, may be postulated. If such substances do appear in the course of glucose utilization, an abnormally high concentration in the blood might well be due to failure of hepatic function, since the liver prob-

ably removes such fragments. The actual substance however, has not been identified in this experiment.

Concerning the primary action of epinephrine in carbohydrate metabolism, most workers are in agreement that it is probably a liberation of the glycogen stores of the animal. (See Cori, 1.)

Lusk (17) exposed completely phlorizinized dogs to cold and found that after a time there was no further excretion of "extra sugar."

Ringer (18), using phlorizinized, deglycogenized dogs found that "extra sugar" did not appear following epinephrine administration, i.e., the D:N ratio remained constant.

The experiments of Lusk, and Ringer, were confirmed and extended by Woodyatt (19), who gave subcutaneous injections of epinephrine every three hours to phlorizinized dogs. After six or eight injections there was no further increase in sugar excretion whether epinephrine was administered or not, and analyses of liver and muscle revealed no glycogen.

On the conception that the primary action of epinephrine is a liberation of the available glycogen the following interpretation of the blood sugar curves may be made.

Epinephrine administration causes hyperglycemia and glycosuria by mobilization of stored glycogen. With the disposal of this mobilized glucose by excretion, by combustion or by conversion into fat, and with the depletion of stored glycogen, there is a decrease in the blood sugar values to normal. Because of the lack of available glycogen, continued administration of epinephrine at even double the original rate does not lead to further hyperglycemia and glycosuria. The sugar furnished by protein breakdown is sufficient to provide for a normal glycemia.

With the sudden cessation of epinephrine injection, the animal quickly replenishes its glycogen reserves at the expense of the sugar in the circulating fluids, causing hypoglycemia. In other words, the rate at which glycogen is stored exceeds for a time the rate at which sugar is supplied to the blood by glycogen mobilization and protein catabolism.

After a period without epinephrine injection, during which the glycogen reserves have been replenished, further administration of epinephrine leads to a second period of hyperglycemia and glycosuria, although not as marked as the first.

It is notable that the total urinary nitrogen excretion as measured by twenty-four hour periods, was not changed by epinephrine injection.

The abrupt hypoglycemia following the cessation of epinephrine administration might be due to an increased insulin secretion. A theoretical correlation of the endocrine glands, including an increased insulin secretion following epinephrine administration has been elaborated by Eppinger, Falta and Rudinger (20). The cross-transfusion experiments of Zunz and La Barre (21) appear to support such a theory. Their criterion of an in-

creased insulin secretion was the appearance of a hypoglycemia in a recipient animal, which was receiving pancreatic blood from a donor injected with epinephrine.

However Colwell and Bright (22), by injecting epinephrine at constant rates into amytalized cats, both alone and with glucose, obtained respiratory data which tended to show a suppression of glucose oxidation. They suggested the possibility that insulin secretion is suppressed under these conditions. That glucose oxidation is not suppressed in unanesthetized dogs can be shown in the present work by the fact that sugar from protein breakdown was not continuously excreted in spite of the constant administration of epinephrine. In addition, Colwell and Bright found that when epinephrine was administered alone, the glycosuria disappeared in twenty-four hours, but that the blood sugar values tended to remain elevated. This difference in results may be due to the use of amytal, since it has been shown that amytal retards the return of blood sugar to normal values.

It is possible that the *persistent* hypoglycemia which occurred toward the end of two experiments (see protocol 3) and which appeared even before the cessation of epinephrine administration, might have been due to liver damage.

SUMMARY

1. Epinephrine was administered intravenously to unanesthetized dogs at constant rates over relatively long periods of time (up to 13 days).

2. Continuous epinephrine administration resulted in a transient hyperglycemia and glycosuria, both of which disappeared in 24 hours. The normal glycemia persisted with continued injection, even when the rate of administration was doubled, and there was no further glycosuria. The cessation of epinephrine administration while the blood sugar values were within normal limits, led to an abrupt hypoglycemia with a slower return of the blood sugar to normal values. With further injection a second hyperglycemia and glycosuria occurred, although not as marked as the first.

3. Continuous epinephrine administration increased the urinary output in every case.

4. Continuous epinephrine injection did not affect the urinary nitrogen excretion as measured by twenty-four hour periods.

5. Comment is made upon the possibility of interpreting the glycemic curves.

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THE EFFECT OF SYMPATHECTOMY ON SEXUAL FUNCTIONS, LACTATION, AND THE MATERNAL BEHAVIOR OF THE ALBINO RAT

With a Description of the Technique of Sympathectomy in the Rat

Z. M. BACQ[1]

From the Laboratories of Physiology in the Harvard Medical School

Received for publication September 24, 1931

The function of the sympathetic innervation of the female sex organs is still largely unknown. Langley and Anderson (1895) pointed out the effects of electrical stimulation of the sympathetic supply: pallor and contraction of the uterus and vagina. The ovaries, however, are also supplied by sympathetic nerves; there is often to be found an ovarian ganglion. The function of these ovarian nerves is unknown. All that we know is that transplanted ovaries in certain conditions still function (see bibliography in Lipschütz, 1924, and Pettinari, 1928).

Cannon and collaborators (1929) showed that in the cat the processes of fecundation, birth and lactation can go on without coöperation of the sympathetic system. In the rat, the estrous cycle (Sweet and Thorp, 1929) and the spontaneous activity (Bacq 1931b), both influenced by the endocrine secretion of the ovaries, are not affected by abdominal sympathectomy.

Following Doctor Cannon's suggestion, I have undertaken a study of the sexual functions, lactation and maternal behavior of the female albino rat after exclusion of sympathetic impulses to the genital organs and mammae.

Technique. All the operations were performed under ether anesthesia. Little care was taken for asepsis. During operations the source of light was a small movable lamp attached to a Leitz standard. I found it possible to perform all the operations described below without the use of a dissecting microscope. One becomes easily accustomed to the small size of the sympathetic system of the rat. The animals used for the experiments reported in this paper were all from Wistar stock.

Abdominal sympathectomy. The skin is incised from the xyphoid process to the pubis. Retractors (weight attached to little hook with a

[1] Fellow of the C. R. B. Educational Foundation.

strong thread), two near the xyphoid and a third near the pubis give a large opening. The intestines are rolled in a cloth, moist with warm water, and drawn to the animal's left side. The peritoneum is dissected between the right ureter and the vena cava. Going deeply behind the vena cava, which is carefully retracted by an assistant, one sees first the femoral nerve, and finally one finds exactly in the middle line the sympathetic chains; they are very small whitish strands, crossing at right angles the little veins coming to the vena cava at the level of each lumbar vertebra. With a small forceps (without teeth) and a small blunt dissector, the chains are now removed from the sacral promontory up to the second lumbar ganglion inclusive. To permit removal of the chains as high as that ganglion, the renal vessels must be lifted with small hooks by the assistant. It may be found necessary to transfer the mass of intestines to the right side in order to remove the third and second left lumbar ganglia. The operation is made rather difficult by the peculiar anatomical disposition of the lumbar sympathetic system of the rat: below the third lumbar ganglion, there is in most cases only one ganglionic mass at the level of each vertebra, but the chains are separate and make a kind of ring around the vessels connecting the spinal cord with the aorta and vena cava. Thus, if the right chain is removed complete with the ganglion, the left chain comes in pieces. Care must be taken to avoid any hemorrhage, because the presence of blood makes it very difficult to distinguish the sympathetic strands. In closing, the abdominal wall and the skin are sewed separately. Wounds heal perfectly in a few days.

After a careful study of the macroscopic anatomy of the sympathetic system of the rat, I thought that some fibres coming from the superior mesenteric plexus and going to the genitalia might have been left uninterrupted by this operation, and consequently I developed the following technique to remove the superior mesenteric plexus. Some sympathetic fibres innervating the ovaries seem to have their origin in that plexus.

Removal of the superior mesenteric plexus. The same incision is made as for abdominal sympathectomy, but the intestines are retracted to the right side. The spleen is drawn out of the abdomen with the stomach after severance of its ligaments. A piece of cotton holds in position the left lobe of the liver. The dorsal peritoneum is incised between the celiac and the superior mesenteric arteries. The left splanchnic nerve is cut high above the ganglionic mass in its course that I have called the adrenal ganglion, and the whole plexus (left adrenal ganglion, and the celiac and superior mesenteric ganglia) is removed in one piece after careful dissection (see fig. 1). The right adrenal ganglion, deeply situated, is generally left in place. The two arteries (superior mesenteric and celiac arteries) and the space between them and the aorta must be thoroughly cleared. The little renal ganglion also may be removed with the connective tissue

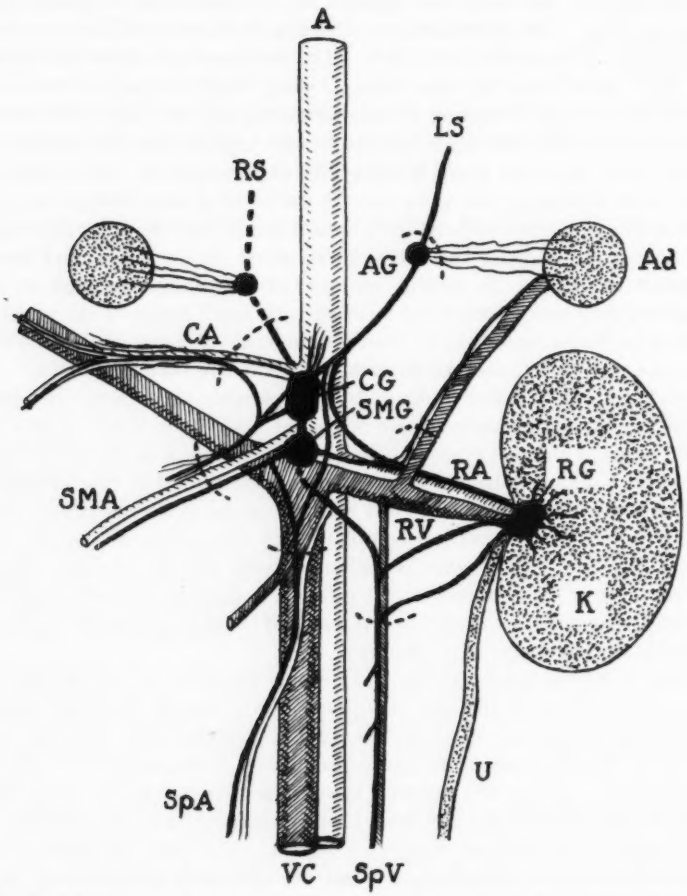

Fig. 1. Schematic drawing showing the general anatomical disposition of the superior mesenteric plexus of a male rat. The intestines are supposed to be retracted to the right side. *A* = aorta; *V C* = vena cava; *Sp A* = spermatic artery; *Sp V* = spermatic vein; *R A* = renal artery; *R V* = renal vein; *K* = kidney; *U* = ureter; *S M A* = superior mesenteric artery; *C A* = celiac artery; *Ad* = adrenal gland.

Sympathetic strands and ganglia are in black: *C G* = celiac ganglion; *S M G* = superior mesenteric ganglion; *R G* = renal ganglion; *A G* = adrenal ganglion; *L S* = left splanchnic nerve; *R S* = right splanchnic nerve. In the female, sympathetic strands, instead of going along the spermatic vessels, follow the ovarian arteries and veins. Interrupted lines indicate where the sympathetic nerves are cut when the superior mesenteric plexus is removed.

covering the renal vein. This operation deprives of their sympathetic innervation the gastro-intestinal tract (except the colon), the abdominal vessels, the left adrenal, the liver, the kidneys, and probably the ovaries. It may be combined with the removal of the abdominal chains. The combination of these two operations is the best technique if one wishes to prevent regeneration and to be sure of sympathetic denervation of the abdomen.

The fact that the anatomy of the sympathetic plexuses of the rat is irregular must be emphasized; figure 1 only shows the most common disposition of this region.

Removal of the thoracic sympathetic chains. Under deep ether anesthesia a small glass catheter is introduced between the vocal cords, and artificial respiration is given through an ether bottle. I used the technique described by Cannon and his collaborators (1929) for thoracic sympathectomy in the cat, but the thorax of the rat must be opened at the second or third intercostal space. The stellate ganglion is deeply situated in the neighborhood of important vessels. The chains may be removed down to the fourth or fifth interspace. To remove the lower thoracic chains, the seventh intercostal space is opened. The lungs must be handled with the greatest care because their tissue is extremely thin.

Abdominal operations are well tolerated by rats. Statistics of thoracic operations show a much greater percentage of death (about 30 per cent). The principal causes are spasm of the vocal cords, injury to the lungs, and deposit of an excess of mucus in the bronchi and trachea.

RESULTS. *General behavior of sympathectomized rats.* Since, to my knowledge, no physiological study (except that by Sweet and Thorp, 1929, on the effect of lower abdominal sympathectomy on the estrous cycle) has been published on the sympathetic system of the rat, it is of interest to report on the general behavior of sympathectomized rats. To be sure, I was unable to prepare a completely sympathectomized rat; but during a long time I kept a few animals with both abdominal chains and one thoracic chain removed, and I succeeded with Dr. M. O. Lee in removing from a male rat all the sympathetic system except the lower left thoracic chain. These animals were in perfect condition—at first sight indistinguishable from normal. They ate well and ran perfectly in revolving cages (Bacq, 1931b). They showed, however, some important deficiencies: Males are impotent after abdominal sympathectomy, because of absence of ejaculation (Bacq, 1931a). Sympathectomized rats are more sensitive to cold. No erection of the hair occurs in the denervated region. (This observation is more obvious in the morning after a rather cold night. Rats sleep with the head between the fore paws, and normal animals are often found in the morning with hair fully erect.) The only case of pneumonia we had to register in our colony was that of a young female who had been

deprived of her sympathetic system with the exception of the left thoracic chain. It was not uncommon to observe sympathectomized males building nests like pregnant females; it is known that the nest building activity may be a reaction to cold (Kinder, 1927; Sturman-Hulbe and Stone, 1929).

Another observation, made incidentally, shows that removal of much of the sympathetic system decreases the resistance to heat. Two rats were exposed on the roof during half an hour at the beginning of a warm afternoon in May, and were tied on the animal board to be photographed. The resulting struggle had no noteworthy effect on the normal animal. But rat 19, from which both abdominal and the right thoracic chains had been removed, exhibited great distress after the exposure to the sun and the struggle on the board. The animal lay on its side, breathing very rapidly, and quite unconscious and insensitive. Taken into the shade the rat recovered slowly, but showed, during several hours, a marked incoördination and a great desire for water. It appeared to be a typical heat stroke.

These observations show that sympathectomy results in a deficient thermoregulation. They confirm the observations of Cannon and collaborators (1929) on cats, dogs, and monkeys.

After removal of the upper thoracic sympathetic chain, rats exhibit a beautiful Bernard-Horner syndrome, i.e., the upper eyelid is much lower than normal, and consequently the opening is obviously smaller. This syndrome disappears during emotional excitement if the adrenals are not inactivated.

Effect of sympathectomy on the estrous cycle, fecundation, gestation and labor. Taking as a test the spontaneous activity, I have not noticed any important change in the estrous cycle of the female rat after removal of both abdominal chains (confirming Sweet and Thorp's findings), or after excision of the superior mesenteric plexus, or both operations combined (Bacq, 1931b).

Sympathectomized females, when allowed sexual relations with normal males, became quickly pregnant. For instance, rat 20 gave birth to a normal litter 22 days after removal of both abdominal chains; the fecundation in that case occurred in the 24 hours following the operation.

I have not observed any peculiar phenomenon during gestation. The vaginal secretion of pregnant, operated females was typically composed of leucocytes, with a few epithelial cells. I have observed the typical blood sign on the thirteenth day of gestation in two sympathectomized females (rats 102–103).

The labor, attentively watched in several instances, was perfectly normal after sympathectomy, with the exception, however, of two suggestive cases:

Rat 101. Second litter after removal of the mesenteric plexus; started parturition and bled to death from the uterus. Autopsy showed the right uterine horn full of

fetuses; the other was empty and looked as if the fetuses had been expelled from it. I am not certain, because the mother possibly ate the young during the absence of the caretaker.

Rat 104. Third litter after operation. Gave birth to 6 young; there was continued slight bleeding from the uterus and *3 days later* she gave birth to 6 more pups. She was bred again at weaning time and gave birth to another litter, again in two batches about 3 days apart, 4 young in the first and 5 in the second batch.

This phenomenon, which is very unusual among normal rats of the Wistar Institute,[2] suggests that sympathetic denervation of the genitalia can have a disturbing effect on labor.

The size of the litter is unaffected by sympathectomy (see table 1). The weight of the young at birth is normal.

Effect of sympathectomy on lactation and maternal behavior. I was soon convinced that the sympathetic innervation of the mammary glands is of little importance. Schäfer says (1898): "Although it is a matter of common experience that the quantity and quality of the milk is in women materially influenced by the condition of the nervous system, the results of experiments upon animals have furnished evidence on this subject which is either entirely negative, or at most of a somewhat conflicting nature." Recently Stricker (1930) has pointed out that transplanted mammae still respond with the usual sensitivity to the sexual hormones and secrete milk after parturition. Two rats from which I had completely removed the right sympathetic chain from the stellate ganglion to the pubis nursed their young normally; no difference could be detected between the right and the left mammary glands; the young suckled indifferently the denervated and innervated mammae.

After abdominal sympathectomy, lactation and maternal behavior are generally normal (see table 1). But twice I have observed the remarkable phenomenon reported by Cannon and Bright (1931): the mammae were not prepared for lactation. My first observation was that on rat 20 (not reported in table 1); it was very striking indeed.

Rat 20. Wistar colony, born 8/8/'29.

December 22/'29. Control litter, nine young, all survived, maternal behavior normal.
Jan. 4/'30. Weight of the litter, 141 grams.
Jan. 13. Weight of nine young, 236 grams, average 26 grams.
Jan. 14. Two abdominal sympathetic chains removed, animal in good condition, weight 200 grams, put in breeding cage.
Feb. 7. In very good health, gave birth to eight normal young, weight of the litter, 32 grams.
Feb. 8. One pup died.

[2] I am indebted to F. Louise Duhring, curator of the animal colony at the Wistar Institute, for valuable information. It is interesting to report that at the Wistar Institute, they have known a litter to be cast during a 24-hour period and also in a 48-hour period, but never during a longer time than that.

TABLE 1

Data on the development of litters of albino rats, before and after various operations on the sympathetic system of the mothers

Control litter

RAT NUMBER	SIZE OF LITTER	YOUNG DEAD AT BIRTH	DIED DURING 5 FIRST DAYS	WEIGHT OF LITTER 13 DAYS AFTER BIRTH	WEIGHT OF LITTER 22 DAYS AFTER BIRTH
				grams	*grams*
17	3	1	0	44	95
23	6	1	2	62	103
13	4	1	1	60	100
18	9	3	0	140	244
19	13	0	0	164	319
101	9	2	0	107	196
103	8	0	1	124	196
102	10	0	4	118	189
104	8	0	4 young eaten	84	140

First litter after operation

RAT NUMBER	PART OF SYMPATHETIC SYSTEM REMOVED	SIZE OF LITTER	DEAD AT BIRTH	DIED DURING 5 FIRST DAYS	WEIGHT OF LITTER 15 DAYS AFTER BIRTH	WEIGHT OF LITTER 22 DAYS AFTER BIRTH
					grams	*grams*
17	Both abdominal chains	6	1	1	91	180
23	Both abdominal chains	11	0	0	174	232
13	Both abdominal chains	9	0	0	124	?*
18	Both abdominal chains	7	0	4	86	143
19	Both abdominal and right thoracic chains	8	1	3	78	164
101	Superior mesenteric plexus	6	0	1	110	131
103	Superior mesenteric plexus	6	0	4	42	74
102	Mesenteric plexus and abdominal chains	10	0	1	139	162
104	Mesenteric plexus and abdominal chains	5	0	0	132	188

Second litter after the operation

RAT NUMBER	SIZE OF LITTER	DEAD AT BIRTH	DIED DURING 5 FIRST DAYS	WEIGHT OF LITTER 13 DAYS AFTER BIRTH	WEIGHT OF LITTER 22 DAYS AFTER BIRTH
				grams	*grams*
19	11	1	1	151	249
101	12**				
103	11	Young in very poor condition. Mother emaciated by diarrhea, nurses them, but their growth is very slow			
102	4	0	2	Took no care of other two young	
104	9	0	1	169	271

* Whole litter killed by the mother. Mammae were fully developed.

** Mother died at parturition from uterine hemorrhage; one uterine horn had expelled its content, the other was full of fetuses.

Feb. 9. Two pups died; mother has no milk, but the maternal behavior is perfectly normal: great nest building activity, takes care of her litter, allows pups to suckle.

Feb. 10. Only two young living. No development of mammae.

Feb. 11. Only one pup living, its weight is 4 grams (same as at birth). Two vigorous young of another litter, nine days old, weighing 12 grams each, given to the mother. She takes care of them; they suckle vigorously.

Feb. 12. Three young found suckling.

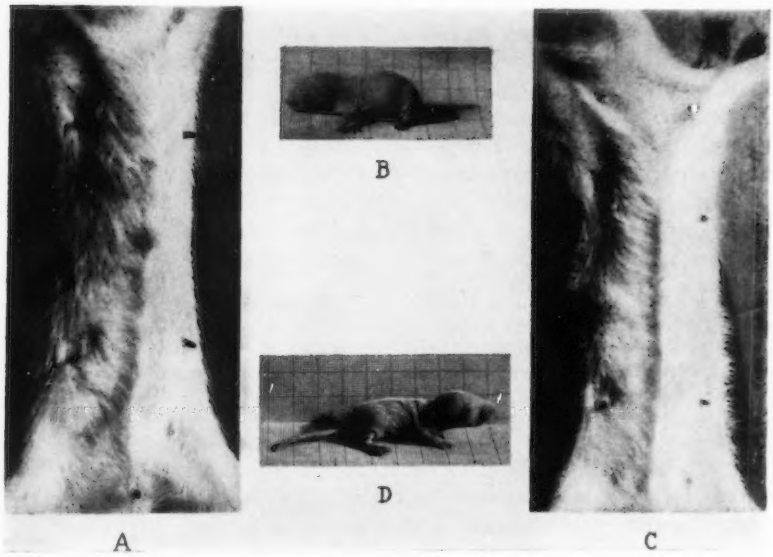

Fig. 2. A. Photograph of rat 20, seven days after parturition, twenty-nine days after abdominal sympathectomy. Notice the aspect and black color of the nipples.

B. The seven days' old pup, the only survivor of a numerous litter; very poorly developed, weight 6 grams.

C. Photograph of rat 20, ten days after parturition; some nipples are necrotic; the anterior pectoral mammae undergo a normal development.

D. The surviving pup when 10 days old.

Feb. 13. Mother in wonderful condition, weight 214 grams. Weight of the two newly brought young, 13 grams. Weight of her sole surviving pup, 5 grams. Blood at the top of five nipples.

Feb. 14. Pictures taken (see fig. 2). Nipples are red and stiff, like little horns.

Feb. 15. Weight of the small pup, 7 grams; the two others weigh 14 grams each, while the weight of two of their brothers nursed by their own mother has reached 20 grams.

Feb. 17. Nipples of four mammae (two abdominal, two pectoral) are necrotic; the other mammae undergo a normal development. Maternal behavior normal.

Feb. 18. Photographs taken, see fig. 2, c, d.
Feb. 19. The black necrotic tissue of the mammae eliminated; weight of surviving pup, 10 grams only.
At this time, four pectoral mammae underwent a rapid development and the weight of the young increased.
Feb. 25. Weight of the pup, 19 grams, while at the same time the average weight of one of the preceding litter of nine pups was 27 grams. (This pup survived and was perfectly normal.) The two other young rats have the same weight as their brothers nursed by their own mother.

This observation leaves no doubt. The mother, though in very good condition was unable to nurse properly her litter; the young were normal, as shown by the fact that one survived; he probably got some milk because of the powerful mechanical stimulation produced by the two vigorous young of another litter. Furthermore, in spite of the anatomical lesions of the nipples, and though having very little milk, the mother allowed the young to suckle. Indeed, it was remarkable to note that her maternal behavior was quite normal. The extent of the lesions suggests that suckling, far from relieving the mother, may have been painful.

Rat 20. Had on March 29, a third litter of 12 young; only two of them died; the others showed a normal development; the mother had plenty of milk and the average weight at the age of 22 days was 30 grams. Our second observation is on rat 102, who did not take any care of her second litter, born after complete denervation of the abdomen; but at that time the animal was not in very good condition.

Thus sympathectomy, in certan obscure conditions, can prevent the normal secretion of milk after parturition. It appears that this effect is not due to the denervation of the mammary glands, but to some trouble brought about in the endocrine government of lactation. No further discussion would be useful at the moment. I merely wish to present the observed facts, for which I have no clear interpretation. It seems, however, that parturition and lactation are not entirely under endocrine control. My observations show that sometimes a non-lactating mother may not take any care of her litter, but also that her maternal behavior may be perfectly normal. The occurrence of these two possibilities, already observed by Cannon and Bright (1931), requires further investigation.[3]

SUMMARY

The techniques of various sympathectomies in the rat are described. Sympathectomized rats exhibit the same deficiencies as other sympathectomized mammals (confirming Cannon and collaborators, 1929). Estrous cycle (confirming Sweet and Thorp's observations), fecundation, and gestation are not affected by abdominal sympathectomy. Deficiencies irregu-

[3] A more complete discussion of the psychological bearing of this observation will appear later.

larly observed after abdominal sympathectomy are the following: the litter is born in two batches a few days apart; the normal milk secretion does not occur after parturition (confirming Cannon and Bright's findings).

No clear interpretation of these facts is possible at the moment Careful observation is reported of the maternal behavior of a Wistar rat during absence of milk secretion.

I wish to express my thanks to Doctor Cannon for his suggestions, his helpful advice and his keen interest in this work. I am also indebted to Doctor Lee for repeated help.

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THE INCREASED METABOLISM OF THE SARTORIUS MUSCLE OF THE FROG FOLLOWING EXPOSURE TO ROENTGEN RADIATION

WALLACE O. FENN AND W. B. LATCHFORD

From the Department of Physiology and the Division of Radiology, The School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

Received for publication September 1, 1931

A survey of the literature relating to the effects of Roentgen rays on tissue metabolism discourages the idea that the effects of such radiation upon the growth or survival of tissues can be attributed to interference with energy yielding reactions. Thus Hubert (1929) found that glycolysis of chick embryos could be inhibited by doses of 6300 r units while 530 r suffices to inhibit growth. Likewise Wels (1924) found that growth of bacteria could be inhibited by doses which leave the oxygen consumption unaffected. Wels and Osann (1924) came to a similar conclusion in regard to yeast.

Nevertheless Roentgen rays do have an effect upon metabolism if applied in sufficiently large doses. Frik and Posener (1926) found that anaerobic glycolysis was more readily affected than respiration, inhibition of glycolysis being observed in testis and retina in doses of 10,000 to 30,000 r or larger. Hubert (1929) also found inhibition of glycolysis in chick embryos immediately after irradiation, 6300 r being the minimum effective dose in his experiments. Both Frik and Posener (1926) and Hubert (1929) measured the metabolism by the Warburg technique immediately after irradiation. Somewhat different results were obtained by Adler (1929) by irradiating the whole animal (rat) and waiting 24 hours or more before killing the animal for metabolism studies. He observed in this way an increase in glycolysis and a decrease in respiration. The highest values were reached 34 to 36 days after irradiation when glycolysis decreases as degeneration set in. Glycolysis may be increased 100 per cent under optimal conditions. These effects were observed with small doses of 600 and 2500 r which are below the minimum effective dose set by Frik and Posener and by Hubert for immediate results. In agreement with these investigators Adler observed no immediate effect with these doses even though some of them were large enough to kill the animal in a few days.

The earliest experiments on the effect of Roentgen rays on oxidation were performed by the dye reduction method. Piccaluga (1923) measured

the reduction of methylene blue by liver tissue and reported that small doses (135 r, a 0.3 skin dose) caused an increase while larger doses (1250 r) caused a decrease in reducing power. Gottschalk and Nonnenbruch (1923) measured the reduction of dinitrobenzol by frog muscle and found an 18 to 38 per cent inhibition by doses of 117 to 1760 r. The dye reduction method however is not altogether satisfactory for the measurement of respiration and Wels (1924) who determined the oxygen consumption of frog muscle manometrically concluded that Roentgen rays had no effect in any dosage up to about 48,000 r (5 hours' exposure). In addition to frog muscle Wels used avian erythrocytes, yeast and bacteria and obtained the same result. His measurements began 30 to 60 minutes after the end of the irradiation and lasted 1 hour. Like Piccaluga, and Gottschalk and Nonnenbruch, he used minced tissue. It appears unlikely therefore that Roentgen rays can have any significant effect upon the oxidizing processes themselves. This is in agreement with the finding of Frik and Posener on mammalian tissues. Adler's finding of a decrease in respiration beginning 24 hours or more after irradiation may be interpreted as a secondary effect rather than a direct effect upon respiration. An apparent contradiction of this conclusion is offered by Roffo and Barbara (1925—read in abstract only) who report a decrease in the respiration of both normal and neoplastic tissue in equal amount. Further details of their experiments are unfortunately not available to us here.

It seems therefore that Roentgen rays have no effect on tissue which is specific for oxidative processes. Any change which does occur (usually found to be a diminution) would seem to be rather the secondary result of some other more fundamental primary cause. Other indications of Roentgen ray effects may be found in changes in acidity (Liechti, 1926), changes in permeability (Kroetz, 1927), decrease in the polarization resistance of the membranes (Waterman, 1928) or increased elimination of phosphorus (from liver not from tumors) (Magath, 1930).

Inasmuch as previous experiments on frog muscle (Wels, 1924) have been performed with minced muscle it seemed worth while to see whether Roentgen rays had any different effect upon intact muscle. There is good reason to expect a different result for the oxygen consumption of the intact muscle is largely dependent upon the "condition" of the muscle and any factor which injures the muscle increases simultaneously its oxygen consumption (Fenn, 1931). Degenerative changes in the nucleus of striated muscle have been seen by Fedder and Hellner (1928) as early as 1 hour after irradiation with doses of 1940 r. Occasional histological study of our muscles fixed and sectioned 6 and 20 hours after radiation confirm this nuclear change. This would lead one to expect therefore an increase in respiration of the intact muscle and we have in fact found a 31 per cent increase after large doses. This again seems to be a secondary

effect of the Roentgen rays rather than a direct effect upon the oxidative mechanism.

Dosage. To simplify the foregoing discussion we have attempted to express all doses in r units as defined by the International Congress of Radiology (Strahlentherapie, xxx, 602, 1928). This has been made possible in an approximate way by the standardization reported by Hubert (1929) who states that with 160 kv and 3 m.a. and 24 cm. distance he gets 105 r per minute without filters. Other doses have been calculated from this relation on the basis that the intensity increases as the square of the voltage and in proportion to the milliamperes and decreases as the square of the distance (cf. Bachem, 1923). This crude method has also enabled us to express our own dosages in r units, pending the accurate measurement of the unfiltered radiation from the Coolidge tube which we used. The intensity of this radiation after passing through copper and aluminum filters has already been measured in skin erythema units. Assuming 450 r units per skin unit (cf. Hubert, 1929) and allowing for the absorption in the filters an approximate estimate of the ulfiltered intensity close to the tube may be made. The result shows that our calculations of dosage from the data of Hubert must be of the right order of magnitude at least. Such calculations, however, cannot be considered to yield more than a rough estimate of the actual doses used particularly since the equality given by Hubert of 105 r units per minute for 160 kv., 3 m.a. and 24 cm. distance gives dosages twice as high as those calculated from a similar equality given by Adler who obtained only 83 r units per minute from 180 kv., 4 m.a. at 23 cm. distance, both without filters mentioned. Since, however, the doses used by different investigators in this field have varied from 100 to 90,000 r units, this regrettable uncertainty regarding the exact magnitude of the dose is relatively immaterial for the present discussion. More serious perhaps is our disregard of wave length and our use of mixed wave lengths since it has been claimed by Moppett (1929) that different wave lengths may have antagonistic effects, a monochromatic beam being more effective than a mixed one of the greater intensity.

Experimental. The oxygen consumption was measured by a differential volumeter (Fenn, 1927) at 22 to 24°C., the temperature being constant to 0.01°C. in any one experiment. In preliminary experiments radiation was applied through the glass walls of the water bath and of the respirometer and through as thin a layer of water as possible. This permitted continuous readings of the oxygen consumption during and after treatment but necessitated a considerable reduction in dosage. Preliminary experiments of this sort with rabbit intestine, frog skin and frog muscle showed no certain effect of any sort and convinced us that there was no great advantage in insisting upon continuous readings during the period of treatment, a procedure which introduced great practical difficul-

ties. Accordingly the following method was adopted and was followed in all the experiments here reported.

Matched sartorius muscles were dissected from each leg of the frog (mostly *R. pipens* and *R. clamitans*) in the morning. After a preliminary soaking in Ringer's solution containing phosphate buffers, to avoid danger of non-irritability, the muscles were inserted, one in each of two respirometers, and the oxygen consumption was measured for a period of 1 or 2 hours. In some of the later experiments more accurate results were obtained by increasing the number of muscles used in each respirometer. After this preliminary period both sets of muscles were removed from the respirometer and were suspended one set in each of two similar test tubes which were closed with rubber stoppers. One test tube was hung close to the Coolidge tube and was irradiated for the stated length of time. The other was left at room temperature for an equal length of time. After treatment both sets of muscles were returned to the respirometers and the oxygen consumption was followed for the remainder of the day. The rates of oxygen consumption before and after treatment were then compared. Except in the last seven experiments the muscles were not in Ringer's solution in the respirometer but were in an atmosphere of pure oxygen. It was accordingly unnecessary to shake the respirometers. The results of the first hour succeeding the treatment were omitted from the final tabulation in the first four experiments because the respirometers used in these cases had unusually large vessels and were consequently particularly slow in coming to equilibrium. Equilibrium is attained somewhat more rapidly however if the muscles are immersed in Ringer's solution in the respirometer. This procedure also keeps muscles in better condition. Care was always taken in successive experiments to alternate the respirometers between the irradiated and the control muscles in order to rule out any possible instrumental differences.

The source of the radiation was a large water-cooled Coolidge tube used for "deep therapy." The muscle was placed 20 cm. from the target in a pyrex test tube without any other intervening filters. The sphere gap showed 200–210 kilovolts and the current was 35 milliamperes (mechanically rectified). The duration of exposure was 1 to 30 minutes. The intensity was calculated to be approximately 3000 r units per minute. The corresponding intensities used by previous investigators were 20 to 30 times smaller than this. Thus Hubert (1929) used an intensity of 105 r units per minute, Wels (1924) 161, Gottschalk and Nonnenbruch (1923) 117, Fedder and Hellner (1928) 129, Piccaluga (1923) 20, Adler (1929) 83, etc. In 30 minutes we could therefore give a dose of 90,000 r units or about 200 erythema doses. The relative insensitivity of the muscles to such enormous doses seems more remarkable than the small increase in metabolism which we actually observed.

RESULTS. The results of these measurements carried out at intervals during the last three years are collected in table 1. The 25 experiments are

TABLE 1

Rate of oxygen consumption of frog sartorius muscles after roentgen ray treatment

DATE	MINUTES OF IRRADIATION	RATE BEFORE IRRADIATION		RATE AFTER IRRADIATION IN PER CENT OF PREVIOUS RATE			
				Irradiated		Control	
		Irradiated	Control	1st hour	2d to 4th hours	1st hour	2d to 4th hours
Muscles without Ringer's solution							
		cu. mm. per gm. per min.	*cu. mm. per gm. per min.*	*per cent*	*per cent*	*per cent*	*per cent*
September 13, 1929	20	0.55	0.81		202		100
September 14, 1929	20	0.93	0.83		83		78
September 16, 1929	20	0.69	0.74		206		108
September 24, 1929	20	0.78	0.76		133		122
September 25, 1929	1	0.67	0.67	98	100	57	91
April 24, 1930	30	0.70	1.26	116	127	138	144*
April 24, 1930	30	0.75	1.00	118	118	101	99
May 8, 1930	20	0.48	0.87	146	127	90	98
May 8, 1930	20	0.51	0.71	149	129	90	99
May 29, 1930	20	0.61	0.52	115	120	90	92
May 29, 1930	20	0.69	0.49	133	114	98	108
June 12, 1930	30	0.47	0.44	176	168	107	127
June 12, 1930	30	0.68	0.69	190	197	72	68
June 16, 1930	5	0.59	1.00	63	73	45	44
June 16, 1930	5	0.47	0.53	83	85	68	72
June 17, 1930	10	0.53	0.57	62	85	81	84*
June 18, 1930	20	0.49	0.60		100		82
June 18, 1930	20	0.84	0.85	67	72	82	99*
Muscles in Ringer's solution							
August 7, 1930	20	0.35	0.53	97	103	83	83
August 7, 1930	20	0.53	0.73	124	117	52	67
July 20, 1931	20	1.05	0.93	120	73	91	61
July 20, 1931	20	0.60	0.64	125	93	91	78
July 18, 1931	20	0.53	0.54	177	131	117	78
July 23, 1931	20	0.39	0.48	116	108	112	81
July 23, 1931	20	0.55	0.60	113	102	84	71
Average		0.62	0.71	119	119	87	89

* See text.

arranged in chronological order. The dosage as measured by the number of minutes of irradiation is given in the second column. The resting rates

of oxygen consumption for the irradiated and the control muscles are given in the third and fourth columns respectively in cubic millimeters of oxygen per gram wet weight of muscle per minute. The average rate of the irradiated muscles before treatment was 0.62 cu. mm. per gram per minute as compared to 0.71 in the control. The accelerating effect of the Roentgen ray treatment was not sufficient to make the rate of oxygen consumption of the irradiated muscle consistently greater than the control nor was it consistently greater than its own rate previous to treatment. This is due to the prevailing tendency of these muscles to fall off during the day in their metabolic rate. It was found, however, that *after treatment the rate of metabolism in the irradiated muscle had either increased or had at least fallen off less than the rate of the control muscle.*

This fact is shown in the last 4 columns of table 1 where the rates found after treatment for the 1st hour and the next 3 hours are expressed in per cent of the rate of the same muscle before the period of irradiation. In the irradiated muscle the rate had increased on the average to 119 per cent while in the control muscle the rate had decreased on the average to 88 per cent. It may be concluded therefore that the radiation caused a 31 per cent increase in metabolism.

Three of the 25 experiments of table 1 differed from the others in showing a higher rate in the control muscle than in the irradiated muscle after treatment. These experiments are marked in table 1 by stars. In the last of these 3 experiments the control muscle had a much higher rate than its fellow before treatment indicating that it was not in good condition to begin with. Such muscles usually show a spontaneous increase in rate during the day anyway suggesting that the injury is spreading or the muscle going into rigor. For the other two exceptions there is no obvious explanation but the metabolism of these muscles especially when they are not kept in Ringer's solution is very susceptible to small variations in their treatment and it is not surprising that a small difference should be masked accidentally in 1 muscle out of 12. The last 7 experiments are the most reliable because the muscles were kept in Ringer's solution and in these cases no exceptions are found.

Comparison of the resting rates of the control and irradiated muscles previous to treatment will show that we were not usually able to dissect out two matched muscles from the same frog in such a way that the metabolism was the same in both. Sometimes this difference was obviously due to a sort of contracture which developed locally at the motor point and which is easily visible to the naked eye. Even when there was no such obvious difference, the higher rate was probably to be attributed to greater stimulation during dissection. When there was a difference between the two muscles in the preliminary period we chose the one with the lower rate for irradiation with the idea that a definite increase due to the radia-

tion would then be of more significance. This was probably a bad policy but it does not appear to affect the significance of the conclusion. The mean difference between the two muscles before treatment was 0.14 cu. mm. per gram per minute or about 20 per cent.

On account of the well known latency in the appearance of Roentgen ray effects, one experiment was tried in which one leg of each of four frogs was irradiated. The animals were then killed successively on the 1st, 2nd, 4th and 8th days after irradiation and the oxygen consumption of muscles from the irradiated and the unirradiated legs were compared. During the irradiation the frogs were placed in a lead box with one hind leg of each frog protruding through a hole provided for it. The frogs were 57 cm. from the target and were irradiated for 92 minutes using 35 milliamperes and 205 kilovolts, without filtration. The results are shown in table 2, and may be interpreted as indicating no significant change in metabolism due to the treatment. Nor were any other differences ob-

TABLE 2

DAYS AFTER IRRADIATION	AVERAGE OXYGEN CONSUMPTION	
	Irradiated	Control
	cu. mm./gm./min.	*cu. mm./gm./min.*
1	1.03	0.98
2	0.67	0.91
4	0.82	0.64
8	0.55	0.68

served between the irradiated and control muscles during the experiment either previous or subsequent to the death of the frog.

It is quite certain that the increased metabolism observed in the experiments of table 1 was not caused by electrical stimulation of the muscle during irradiation. The test tube containing the muscle was suspended by a string about 50 cm. long so that it was well insulated. The muscle was completely enclosed by the test tube with no metal of any sort near by to act as an antenna. There was therefore no real possibility of a contraction in spite of the proximity of the muscles to the high tension leads of the Coolidge tube. It was of course unsafe to be sure of this point by continuous personal observation of the muscles during the treatment but in one experiment we tied the muscle to a small celluloid scale inside the test tube and so measured the length of the muscle after 1, 10 and 20 minutes of irradiation during brief intermissions in the treatment. There was not the slightest suggestion of a shortening to be seen. Since there was no weight to extend the muscle any contraction would surely have been detected in this way. In our experience muscles attached to the usual

metal clamps, stands and levers always contract when placed near the high tension leads of a Roentgen ray tube, so that a graphic record could not be obtained without special arrangements.

We feared at one time that the increase in metabolism which we observed might be attributable to the rise in temperature of the muscle during its treatment. Under certain conditions a 20 minute exposure to a temperature only a few degrees higher than the room temperature may lead to a permanent increase in metabolism because of the resulting accelerated onset of rigor mortis. In our last 5 experiments we were able to rule out this possibility very definitely by observing the temperature inside the test tube, as indicated on a mercury thermometer, after each 10 minutes of irradiation and arranging to keep the control muscle at the same or a 1°C. higher temperature. Usually the temperature of the muscles increased 1°C. for each 10 minutes of irradiation. The Coolidge therapy tube was installed inside a large box where in summer the temperature rose to over 30°C. and on one occasion to 33.5°C. The control muscle showed however that the increased metabolism was not due to heat but to the Roentgen radiation.

The increase in metabolism which we have observed is not a transient phenomenon but persists as long as measurements were continued, in one case for 17 hours after irradiation. No other changes were evident in such casual observation as we made either in contractility and irritability or in the onset of rigor mortis. We cannot therefore confirm the finding of Kushner (1925) that the irritability of the frog gastrocnemius muscle is slightly increased by irradiation. We did not, however, make systematic observations of irritability and Kushner used the much smaller dose of about 60 r compared to our maximum dose of 90,000 r.

We wish to acknowledge our indebtedness to Dr. S. L. Warren and Mr. F. W. Bishop of the Division of Radiology for advising us concerning the technique of the irradiation and for their cooperation in some preliminary experiments.

SUMMARY

There is a permanent 31 per cent increase in oxygen consumption of frog sartorius muscles in vitro following intense irradiation with high voltage unfiltered Roentgen radiation. This is probably the secondary result of injury to the muscle rather than a specific effect upon oxidation.

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VARIATIONS IN THE BLOOD OF RABBITS FROM BIRTH TO MATURITY[1]

M. M. KUNDE,[2] M. F. GREEN, E. CHANGNON AND E. CLARK

From the Hull Physiological Laboratory of the University of Chicago

Received for publication September 14, 1931

In our study of changes in the blood of experimental cretin rabbits,[3] it became necessary, from time to time, to make comparisons of the cretin blood with that of their normal litter mates. These comparisons indicated significant and progressive changes in the blood of normal rabbits as age advanced, at least up to the time of maturity. This paper presents our findings in the study of the blood of normal rabbits.

METHODS. The rabbits in our colony represent a mixed population, bred in our own laboratory and to which no new stock has been added for several years. The young nursed their mothers until the weaning time. After weaning the dietary consists of a good grade alfalfa hay, unrolled oats and water *ad libitum*. Generous amounts of carrots or lettuce are added to the dietary daily or on alternate days. The laboratory environment includes adequate hygienic conditions, such as steam heated, well ventilated spaces, but only diffuse sunlight.

Observations on the hemoglobin are reported in terms of grams per 100 cc. The Newcomer method (1923) for determining hemoglobin was employed, the standard being a Bausch-Lomb calibrated colorimeter disc. The red blood cells per cubic millimeter are determined according to the standard Thoma-Zeiss technique. Measurements of the red blood cells was accomplished by a method which we devised to eliminate distortion of the cells as is caused by friction in the smear technique, and has advantages over the method described by Pyper (1924) in that individual cells are measured, thus obtaining the range of variation in size instead of the average size only. Moreover, the criticism has been directed against Pyper's method because a blood film does not make a perfect defraction gradient. The technique which we devised is as follows: A small amount of freshly shed blood is drawn into the diluting pipette of the counting set and diluted

[1] This investigation was aided in part by grants to the University of Chicago from the Rockefeller Foundation.

[2] Seymour Colman Fellow in Physiology.

[3] Preliminary report. This Journal, 1926, lxxvi, 225.

with previously defibrinated serum from the same animal. A thin glass cover slip is placed over the ruled surface of the Thoma-Zeiss chamber and a small drop of blood from the diluting pipette admitted under the cover slip by capillary attraction. The counting chamber is then placed on the stage of a microscope, fitted with an oil immersion lens and a camera lucida. The projected cells are outlined with a fine pencil sharpened out to a long cone (thus eliminating shadow of the pencil). The actual size of the cells thus outlined may then be determined from a rule made by projecting a micrometer scale with the camera lucida, set at the same angle and side arm length (the lens system of the microscope remains unchanged). The total volume of the red blood cells was determined by Sundstroem and Bloor's (1920) modification of the hematocrit method. Blood was obtained from the ear vein in all rabbits three weeks of age and older, and from the external jugular in the newborn.

The total blood volume was determined essentially after the method of Keith *et al.* (1915) with these modifications: ½ cc. of brilliant vital red (1 per cent solution of the dye in 0.8 NaCl) per 500 grams-body weight of rabbit was injected into the marginal ear vein, and after 3 minutes sufficient blood was let from the marginal ear vein of the opposite side to provide serum for two colorimeter readings. This was allowed to form a soft clot which was gently broken and separated from the serum by centrifugation for several minutes at slow speed. One-half cubic centimeter of this serum was transferred to a 10 cc. calibrated flask, diluted to the mark and read against a standard 1:1000 dilution of the dye solution injected (the standard containing the serum equivalent of the unknown). These figures give a higher concentration of the dye in the plasma of the animal than was originally advocated by Keith (1915). Our reason for using the greater amounts of dye is that in our experience, a higher concentration of dye in the plasma resulted in better check readings. Both Keith *et al.* (1915) and Hooper *et al.* (1920) have shown that concentrations of the dye as used by us are non-toxic. Before injecting the dye, an amount of blood equivalent to the dye solution to be injected was let from each animal. Hooper *et al.* (1920) found that the amount of dye removed in the process of clotting varied from 0 to 7 per cent in various samples of dye obtained from different manufacturers, thus making it impossible to use certain dyes unless oxalated plasma was used for the unknown. We made a number of preliminary tests with our sample of dye[4] (Dye from the same sample was used for all experiments of this report) and found that equally good checks were obtained with serum against oxalated diluted plasma, as with oxalated diluted plasma against oxalated diluted plasma. We therefore felt justified in using serum which simplified the calculation to the following formula.

[4] "Vital New Red." G. Grübler & Co.

TABLE 1

Showing progressive changes in the hemoglobin, red blood cells, reticulocytes and hematocrit readings of the normal rabbit from birth to maturity

AGE OF RABBIT	NUMBER OF RABBITS STUDIED AT THIS AGE	HB. (GRAMS PER 100 CC. BLOOD)			R.B.C. (MILLIONS PER CU. MM.)			RETIC. AS PER CENT OF R.B.C.			HEMATOCRIT READINGS		
		Av.	H	L	Av.	H	L	Av.	H	L	Av.	H	L
1 day	14	15.5	19.5	13.0	4.81	5.04	4.37	35.4	39.0	23.0	36.0	40.0	27.3
3 days	9	14.4	15.4	12.4	3.8	4.14	3.56	16.2	20.0	10.0	24.6	25.0	24.2
1 week	10	10.9	11.9	8.0	4.2	4.49	3.92	8.7	10.1	6.2	24.6	25.0	24.2
2 weeks	21	9.8	12.4	8.9	4.1	5.90	4.10	8.0	8.9	7.2	25.3	26.0	24.4
3 weeks	15	10.2	12.4	9.0	5.2	6.0	4.5	7.6	8.0	7.3	25.8	26.0	25.0
4 weeks	7	10.4	11.3	9.5	5.6	5.7	5.5	7.6	8.0	7.3	25.8	26.0	25.0
5 weeks	7	11.6	12.5	11.4	5.7	5.8	5.4	7.6	8.0	7.2	26.9	27.1	25.7
6 weeks	8	10.6	10.8	9.5	5.3	5.8	5.1	8.0	8.2	7.0	26.5	27.0	25.0
7 weeks	10	11.0	12.0	10.6	5.9	6.0	5.6	6.0	7.0	5.5	26.0	27.2	25.0
8 weeks	4	11.7	12.0	10.7	6.0	6.0	5.6	6.0	7.0	5.7	26.0	27.2	26.9
9 weeks	10	11.2	12.0	11.0	6.1	6.0	5.4	5.0	6.2	5.7	27.0	27.4	26.8
10 weeks	9	11.0	12.2	10.0	5.8	6.2	5.0	5.0	3.0	6.0	27.2	27.4	26.9
11 weeks	4	11.9	12.2	10.0	5.7	6.1	4.7	5.3	6.0	4.2	26.0	28.0	25.0
12 weeks	5	11.4	11.6	11.2	6.2	6.1	5.1	5.0	6.0	4.2	27.4	28.0	27.0
13 weeks	6	11.4	11.6	11.2	6.2	6.2	5.2	5.1	6.0	4.2	27.4	28.0	27.0
14 weeks	6	11.9	12.8	11.6	6.3	6.4	5.8	5.1	5.9	4.3	28.0	24.0	27.0
15 weeks	3	12.6	13.4	12.0	6.6	6.9	6.0	5.0	5.9	4.3	28.0	30.7	27.0
16 weeks	7	13.3	14.0	12.6	6.7	7.0	6.3	5.0	5.6	4.0	29.9	30.7	28.0
17 weeks	6	14.0	15.0	13.0	6.7	7.5	6.5	3.0	5.6	4.0	30.0	30.7	28.0
18 weeks	6	14.2	14.0	13.6	7.0	7.5	6.5	3.5	3.6	3.1	31.0	31.7	28.5
19 weeks	2	14.0	14.3	12.0	7.0	7.5	6.0	2.1	4.1	1.1	31.0	31.7	28.5
20 weeks	7	14.2	14.4	12.6	7.0	7.5	6.2	3.1	4.2	1.0	31.0	31.6	28.2
21 weeks	9	13.9	14.6	12.8	7.1	7.3	6.4	2.4	3.2	1.0	31.0	31.6	28.1
22 weeks	10	13.9	13.75	11.7	7.0	6.7	5.9	2.2	3.1	1.0	31.0	32.0	27.9
23 weeks	10	13.8	14.0	13.6	6.8	6.7	5.9	2.0	2.1	1.0	30.0	32.0	27.9
24 weeks	6	13.4	14.0	13.0	6.3	6.7	5.9	2.1	2.4	1.6	30.0	32.0	27.9
25 weeks	5	12.8	14.0	12.0	6.3	7.0	6.8	2.1	2.2	1.6	29.0	31.4	27.0
26 weeks	5	12.7	13.9	12.0	6.8	7.0	6.5	2.0	2.8	1.6	28.1	31.0	27.2
27 weeks	4	12.7	13.9	12.0	6.6	6.8	6.0	2.0	2.8	1.6	28.4	28.9	28.0
28 weeks	6	12.8	14.0	12.0	6.5	7.0	6.0	2.0	2.1	1.9	28.4	29.0	28.0
29 weeks	4	12.7	13.4	12.2	6.6	6.7	6.1	2.4	2.0	1.8	28.4	29.0	28.0
30 weeks	8	12.8	13.0	12.0	6.5	6.5	6.0	2.0	2.4	1.8	28.0	28.6	27.8
31 weeks	4	12.8	13.2	12.4	6.5	6.6	6.2	2.0	3.1	1.9	28.0	28.6	27.8
32 weeks	7	12.8	13.8	12.0	6.5	6.8	6.1	1.7	1.9	1.6	27.9	28.6	27.0
33 weeks	5	12.6	13.1	12.2	6.4	6.5	6.2	2.0	2.4	1.0	28.0	29.0	27.8
34 weeks	7	12.9	13.4	12.6	6.3	6.8	6.3	2.7	2.6	1.0	28.4	28.6	28.0
35 weeks	6	12.8	13.6	12.0	6.3	6.7	6.0	2.9	3.5	1.7	28.0	29.0	27.0
32 weeks	6	12.6	13.2	12.0	6.2	6.5	6.0	2.0	2.4	1.9	28.0	29.2	27.0
37 weeks	6	12.7	13.9	11.8	6.2	6.9	5.8	1.8	3.0	1.6	28.4	29.8	26.9

Av. = average; H = highest; L = lowest; Retic. = reticulocytes.

With the standard set at 15, to obtain the total dilution *"The reading of the unknown X1000"* = Total dilution.

$$\frac{\text{Total dilution of dye injected} \times \text{No. of cc. of dye solution injected}}{20 \times 1 - (\%\ \text{corpuscles of whole blood})} = \text{cc. of blood in the animal.}$$

Reticulocyte counts were made by making thin blood smears on cover slips previously prepared with 0.3 per cent water solution of brilliant cresol blue and counterstaining with Giemsa's stain; 1000 or more cells were counted before estimations were made.

RESULTS AND DISCUSSION. Data presented in table 1 of this paper and charted in the paper dealing with blood changes in hyper- and hypothyroidism (Kunde *et al.*, 1931) show numerical values and illustrate graphically the progression of changes which occur in the blood of normal rabbits from birth to maturity.

Hemoglobin and number of red blood cells. In the rabbit the highest average amount of Hb (15.5 gms.), range in variation = 13 to 19.5 grams, occurs within 24 hours after birth. This is followed by a sharp drop during the first 3 days and a more gradual decline into the 2nd and 3rd weeks after birth. Contrary to this, the red cell count is low at birth (4.5 to 5 million) but falls to a still lower level about the second week after birth and then a steady rise; the peak of the curve (7 million) being attained about 16 to 18 weeks after birth. The hemoglobin at this time is approximately 14 grams, i.e., about 2 grams Hb for every million red blood cells. In some few instances, a maximum of 7 million was found as early as the 8th week. The high count of 7 million holds fairly constant up to the 6th or 7th month. A perceptible fall to 6 or 6.5 millions may then occur. The count varies slightly about this level throughout the remainder of adult life provided the animal is in good nutritional state. We add this last statement because we have frequently noticed a decrease of one million or more red blood cells associated with upper respiratory infections, excessive summer heat or the elimination of alfalfa, carrots or lettuce from the dietary. The concentration of hemoglobin, which is approximately 3 grams per each million cells at birth, falls, by the end of the 2nd week of post-natal life, to approximately 2 grams per million cells and holds that ratio quite constantly throughout the remainder of adult life.

Williamson (1916) studying the effect of age on the hemoglobin per cent in man, finds about twice as many grams per hundred cubic centimeters blood at one day after birth as at the end of the first year. No corresponding red cell counts were made in connection with his studies. Williamson *et al.* (1926) report that in the rat the Hb steadily falls during the first 50 days of life and then gradually rises reaching a maximum about the 150th day. Sure, Kirk and Walker (1928) report on both hemoglobin

concentration and number of red blood cells in the albino rat from birth to the 345th day. The erythrocytes show a constant increase up to the 75th day. The Hb increases very slightly for the first 5 days, then drops continuously for about two weeks and holds at its lowest level through the third week. Then rises constantly reaching a maximum on the 75th day.

The reticulocytes at birth constitute 23 to 39 per cent of the total red blood cells. At the end of three days the number falls to between 10 to 16 per cent of the red blood cells. At three weeks they average 10 per cent of the erythroyctes. From then on there is a slow gradual drop until about the 15th week when values from 0.5 to 3 per cent are found on through the 9th month of life.

The corpuscle plasma ratio averages 36 per cent at birth, the range of variation being between 27.3 and 40 per cent. At the end of 2 weeks 23 to 26 per cent of the whole blood (by volume) consists of corpuscles. Following this the per cent erythrocytes increase so that when the peak of the adult hemoglobin curve is reached the hematocrit readings vary between 28.3 and 31 per cent (av. = 29.4). After a few weeks a slight decrease coincident with the drop in erythrocytes occurs so that the reading varies between 29 and 26 per cent.

The size of the red cells. Several hundred measurements were made within 12 hours after birth. These vary between 9.5 to 11 μ, the average being 10 μ. Five to six months later the size of the cells varies between 8 and 9 μ, the adult average being 8.5 μ.

The total blood volume was determined on the 3rd, 5th, 12th, 16th, 19th and 24th weeks. The highest total blood volumes were found at 3 weeks after birth. These vary between 9 and 9.67 per cent of the body weight. From the 5th to the 28th week the results fall between 6.68 and 8.2 per cent of the body weight. These slightly higher total blood volumes of the growing young rabbit correspond to the findings reported by Cartland and Koch (1928). However, our work does not suggest that age *per se* has a marked influence on the total blood volume since differences of 1.5 per cent or more were found in litter mates. This suggests that perhaps the nutritional condition rather than the age is the determining factor in causing these variations. Wendt and Drinker (1929), studying the blood volume of rabbits weighing 2000 to 3200 grams, find variations in that group of adults which equals the variations in our entire age range of 3 to 28 weeks. The average blood volume reported by these investigators is 8.7 per cent of the body weight.

SUMMARY

In a normal rabbit the highest hemoglobin per cent occurring during extra-uterine life is found within 24 hours after birth. Associated with

these are: 1, a low erythrocyte count (4.5 to 5 million); 2, a high reticulocyte count (35 per cent or more of the erythrocytes); 3, hematocrit readings averaging 36 per cent, and 4, large red cells 9 to 11 μ in diameter.

Two to three weeks after birth, the Hb. per cent and erythrocyte count have reached their lowest levels. The maximal hemoglobin per cent (14 gms. per 100 cc. of blood) with hematocrit readings averaging 31 per cent, and diameter of erythrocytes falling between 8 and 9 μ (av. 8.5 μ); the reticulocytes being 0.5 to 3 per cent of the red blood cell count, occurs at maturity. These values hold for approximately 2 mo., after which a slight decrease in all values is again apparent.

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BLOOD CHANGES IN EXPERIMENTAL HYPO- AND HYPER-THYROIDISM (RABBIT)[1]

M. M. KUNDE,[2] M. F. GREEN AND G. BURNS

From the Hull Physiological Laboratory of the University of Chicago

Received for publication September 14, 1931

Kocher (1883) indicated that the thyroid gland exerts a specific effect upon the cellular content of the blood. Crotti (1922) and Mackenzie (1926) reviewed the literature on this subject and added their findings. Crotti emphasizes the fact that anemia may be associated with myxedema, and notes that in hyperthyroidism the number of red blood cells and the hemoglobin content of the blood are usually normal; that in young women with hyperthyroidism the number of red blood cells may occasionally be greater than normal. Mackenzie notes that hyperthyroidism may be characterized by an anemic condition, but that anemia is more often associated with hypothyroidism.

This lack of unanimity reported in blood findings of clinical hyper- and hypothyroidism led us to attempt a systematic examination of the blood of rabbits of known stock on a carefully controlled diet, under conditions of experimentally induced hyper- and hypothyroidism. Previous to our preliminary publication (Kunde, 1926) Esser reported on the blood studies of 2 rabbits with complete extirpation of the thyroid glands. Other than this, we were unable to find evidence of animal experimentation in which the thyroidectomy did not also involve the parathyroid glands.

EXPERIMENTAL PROCEDURE AND METHOD. The technique of producing experimental cretinism in the rabbit has previously been cited (Kunde, 1927). Suffice it to say here that the thyroid glands of animals in this study were all extirpated between the 2nd and 3rd weeks after birth.

Hyperthyroidism was induced in both normal and cretin rabbits by feeding 250 mgm. to 450 mgm. desiccated thyroid in gelatin capsules daily. Blood examinations of the cretin rabbits were made at weekly intervals (fig. 1 presents a graphic comparison of these results, to weekly examinations of the blood of normal rabbits) (Kunde et al., 1931). The hyperthyroid group was studied at irregular periods. The same methods were employed in determining the total blood volume; Hb. per cent; volume

[1] This investigation was aided in part by grants to the University of Chicago from the Rockefeller Foundation.

[2] Seymour Colman Fellow in Physiology.

ratio of corpuscles to plasma; count and size of the red blood cell; and per cent reticulocytes, as has already been described. The cholesterol determinations were made according to Meyers and Wadell (1918) technique.

EXPERIMENTAL CRETINISM. *The red blood cell count.* No significant change in the erythrocyte count was observed until 4 or 5 weeks after extirpation of the thyroid glands. The red blood cell count in the cretin

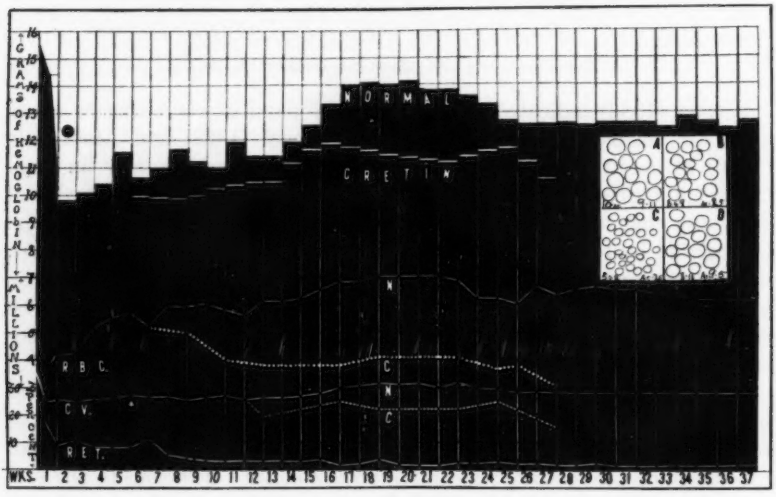

Fig. 1. Graphic presentation of progressive changes occurring in the blood of normal and cretin rabbits from birth to 37 weeks of age. The horizontal columns indicate weekly intervals. The black background shows weekly changes in the hemoglobin of normal rabbits. The scale = grams Hb. per 100 cc. blood. The black dot indicates the time of thyroidectomy. The uppermost white line on the black background indicates the onset and course of deviation of the cretin hemoglobin from the normal. The scale for the red blood cells (4–5–6–7) = millions per cubic millimeter. *N* = normal, *C* = cretin. *C. V.* = per cent by volume of the cells in the whole blood; the scale 10–20–30 = hematocrit readings. *R. E. T.* = per cent reticulocytes of the red blood cells in the normal rabbits. The scale is the same as hematocrit readings. The insert (drawn to scale) indicates changes in the size of the r.b.c.; *A* = cells at birth, 9 to 11μ. av. = 10μ. *B* = size of cell at 7 months = 3–9μ. av. 8.5μ. *D* = cells 12 weeks after thyroidectomy = 8–11μ. Av. 9.5μ.

then numbered approximately 5 million, as against 6 million for the normal unoperated rabbit of the same age. The decrease in erythrocytes continues rather sharply so that at the age of 11 weeks the count has fallen to 4 million and holds at approximately that level through the succeeding 10 to 12 weeks. This is through the age range that the red blood cell count in the normal rabbit has reached 7 million (see fig. 1). Following this, the

anemia increases, reaching its greatest severity just before death of the animal, which may be 4, 5, or 6 months after thyroidectomy. Those that survive run a red blood cell count of approximately 3 million.

The hemoglobin in the cretin diminishes also, but correspondingly less than the diminution in the number of red blood cells. Consequently a high color index is a characteristic finding. Normally the adult rabbit's blood contains very close to 2 grams hemoglobin per million erythrocytes. But in the cretin (8 to 10 weeks after thyroidectomy) the hemoglobin runs very close to 3 grams per million red blood cells, and frequently just before death even a higher concentration has been noted. This high color index first led us to suspect a close similarity of this anemia to pernicious anemia. However, as our study progressed we found a lack of parallelism between other important conditions in these anemic rabbits and the clinical entity which characterizes pernicious anemia, viz., the aciditiy of the gastric contents in the cretin is normal, (Green and Kunde, 1930) and the bone marrow does not present the picture found in pernicious anemia, as will be fully described later.

Moreover, it has been frequently noted (Evans, 1926) in long standing secondary anemias, especially those associated with acute infections, that the color index, while commonly not as high as in pernicious anemia, is greater than 1. We were unable to locate chronic infections in the cretins but the duration of the anemia is usually 10 weeks or more before the color index becomes significantly higher than normal. This is an appreciable portion of the life span of the rabbit. (An attempt was made to ascertain the longevity of domestic rabbits. The greatest ages reported were 13 and 11 years.)

Evans (1926) points out that a color index over 1 merely indicates a disturbance in erythropoietic activity greater than the disturbance in the hemoglobin metabolism although both may be involved.

The cholesterol content of the cretin rabbits with severe anemia varied from 175 mgm. to 190 mgm. per 100 cc. whole blood. This is more than 100 per cent over the concentration of cholesterol in the whole blood of the control litter mates (variation between 67.7 and 82.9 mgm. per 100 cc. whole blood were found in the normal animals). Here again is an outstanding difference between the anemia of hypothyroidism and pernicious anemia. Bloor and Knudson (1918) and Gorham and Mayers (1917) have shown that in pernicious anemia there is a diminution of the total cholesterol in the whole blood. Harnes (1929) shows variations in the cholesterol content of the blood of rabbits due to different environmental conditions of light. This can be excluded as a causative factor in our rabbits since both cretin and control animals lived in diffusely lighted laboratories at all times. Kanner (1926) points out that obstructive jaundice in man and hemolytic icterus in dogs is accompanied by a high percentage of cholesterol in the

blood, when there is an accumulation of bile pigments in the Kupffer cells. Since no examination of the Kupffer cells in these animals has been made, we do not know whether an analogous condition exists here or not. Careful study of the liver in hyper- and hypothyroidism should be made.

The bone marrow in experimental cretinism was examined by Esser (1907) and by Tatum (1913). Esser finds the marrow rich in erythropoietic activity, and no essential difference between the red blood cells of the normal bone marrow and the red blood cells of the marrow of the thyroidectomized animals. The giant cells were fewer in number. Tatum found "A fatty inactive cretin's marrow with leucogenetic centers predominating over the erythrogenetic centers. The meyalokarocytes were few and degenerate." Wm. Bloom (Dept. of Anatomy, University of Chicago) using Maximow's (1923) hematological technique (hematoxylin-eosin-azure II) examined sections of the bone marrow of our rabbits. Bloom found no resemblance of this cretin marrow to the marrow in pernicious anemia. The cretin marrow being a fatty marrow with finely granular purple staining masses occupying the spaces between many of the fat cells. In the center of these are some groups of blood forming cells. The red blood cells are few in number, most of the cells being myelocytes with very few megaloblasts. There are few plasma cells. Some areas are suggestive of what Selling (1911) found after benzol poisoning. Only a few stem cells were present and few reticular cells. Despite this, some areas in each section contained apparently normal bone marrow. This does not conform to the bone marrow of pernicious anemia, which Conheim (1876) (quoting Evans, 1926) described as a raspberry-colored jelly-like mass; histologically containing macrophages and a great number of megaloblastic cells. The cretin marrow, on the whole, resembles rather closely the bone marrow described by Doan, et al. (1925) in starved pigeons.

The size of the red blood cells in chronic hypothyroidism (18 to 20 weeks after thyroidectomy) vary from 8 to 11 μ, the average size being 8 to 9 μ. In the normal rabbit of comparable age the average size is 8.5 μ. The increase in the size of the r. b. c. with increase in hemoglobin content has been described by Capps (1903).

The volume ratio of corpuscles to plasma is less than that of the controls but somewhat greater than would be proportional to the lowered cell count. The increased size of the cells is probably the explanation of this (fig. 1, insert).

The total blood volume of these cretin rabbits indicates that the anemia is not due to an increase in plasma volume, inasmuch as the number of cubic centimeters of blood per gram of body weight falls within the range of variation found in the control littermates.

The per cent reticulocytes in some cretins was slightly higher than their control littermates for the first weeks after thyroidectomy and then lower.

The average, however, runs very close to that of the normal rabbit of the same age; for this reason no graphic record is included in figure 1.

EXPERIMENTAL HYPERTHYROIDISM. Data presented in table 1 gives evidence of the blood changes which occur in both cretin and normal rabbits after the daily ingestion of desiccated thyroids. These data indicate that in both cretin and normal rabbits, certain definite changes consistently occur as the result of thyroid ingestion. The time required to induce these changes varies appreciably in both groups of animals. In all cases there is an initial polycythemia accompanied by a proportionate rise in hemoglobin. But if the hyperthyroidism is continued there invariably results an anemia characterized by a reduction in the number of erythrocytes to 1 or 2 million per cubic millimeter below the count for normals of the same age, and a correspondingly great or greater reduction in the hemoglobin, so that the anemia of prolonged hyperthyroidism is accompanied by a color index of 1 or less than 1. This seems to indicate that in this type of anemia the disturbance in hemoglobin metabolism is greater than the disturbance in erythropoietic activity. As the hyperthyroidism increases in severity there is a diminution in the size of the cells so that frequently only cells ranging between 5 and 8 μ were found (see fig. 1).

Lim (1922) and others find evidence of a stimulating effect on the bone marrow as the result of thyroid ingestion. We also found histological evidence of an increased activity of the bone marrow, the marrow being less fatty than normal, and an increase in the number of red cells and many eosinophiles and myelocytes with few stem cells and few reticular cells. This increased evidence of activity of the bone marrow, even after the disappearance of the initial polycythemia in the blood stream, seems to indicate an increased destruction of the blood cells as the severity of the hyperthyroidism progresses. This phase of the work needs further investigation.

The total blood volume in severe hyperthyroidism shows no reduction below that which one frequently finds in normal thin animals (Dye method). In some rabbits, the number of cubic centimeters of blood per gram of body weight after several weeks of severe hyperthyroidism was almost identical to the figures obtained in the same animal before the ingestion of the thyroids. Others showed a slight decrease and still others a slight increase. We found that in many of our normal thin animals the total blood volume was slightly less per gram body weight than in the fatter ones, and believe that the slight variation obtained can be explained as a function of the nutritional state rather than the hypo- or hyperthyroidism *per se*.

The reticulocyte response to thyroid feeding is quite different in the cretin from what was observed in the normal rabbits fed 250 to 450 mgm. of thyroid daily. Either no increase in the per cent reticulocytes in the peripheral blood stream occurs under these conditions, or at most only a 2 to 4 per cent rise. In the cretin, made hyperthyroid by the daily in-

TABLE 1

Showing the effect of daily ingestion of 250 to 450 mgm. desiccated thyroid in the blood of normal and cretin rabbits

ANIMAL	AGE	CONDITION OF ANIMAL	R.B.C.	HB.	V.R.	RET.	REMARKS
	days						
1	130	Normal	640	12.0		1.5	Beginning thyroid feeding
	156		714	11.4		2.2	26th day; highest R.B.C.
				11.5			
	161		520	10.0		2.5	31st day; lowest Hb. Died. Hyperthyroid
2	119	Normal	562	10.3	27.4	2.8	Beginning thyroid feeding
	159		764	11.9	31.0	6.0	40th day. Highest R.B.C. and Hb
	193		540	10.0	23.7	2.0	74th day. Lowest R.B.C.
3	133	Normal	592	12.3	28.9	3.0	Beginning thyroid feeding
	157		775	13.6	32.0	2.0	24th day. Highest R.B.C. and Hb
	173		510	10.0	26.0	0.1	40th day. Lowest R.B.C. and Hb. Died. Hyperthyroid
4	198	Normal	650	13.6	31.8	2.2	Beginning thyroid feeding
	216		791	14.5		1.2	18th day. Highest R.B.C. and Hb
	251		543	10.4	26.0	0.6	53rd day. Lowest R.B.C. and Hb. Died. Hyperthyroid
5	237	Cretin	426	11.6	23.4		Beginning thyroid feeding (475 mgm.)
	262		638	12.3	33.1		25th day. Highest R.B.C.
					33.6		
	287		643	11.6			50th day. Stopped thyroid feeding
6	263	Cretin	200	7.2	19.2		Beginning thyroid feeding (275 mgm.)
	336		652	12.7	31.4		73rd day
	359		450	9.1	26.2		96th day. Stopped thyroid feeding
7	127	Cretin	455	9.8			Beginning thyroid feeding 200 mgm.
	145		675	14.4	33.0		18th day thyroid feeding
	200		520	10.3	26.0		73rd day. Stopped thyroid feeding
8	239	Cretin	375	10.8	19.0		Beginning thyroid feeding
			590	12.2	28.0		22nd day. Stopped thyroid feeding

R.B.C. = red blood cells; HB. = hemoglobin; V.R. = volume ratio of corpuscles to plasma of whole blood; RET. = reticulocytes.

gestion of 250 mgm. desiccated thyroid, the reticulocyte response varies from 5 to 10 per cent, reaching the peak point about the 10th day of hyperthyroidism, and then subsiding.

Adams and Sherket (1929) show that in the normal rat, on the 4th to 8th day of thyroid ingestion, 34 per cent of the red blood cells may be reticulated cells. This specie difference in response to the thyroid is extremely interesting, since in the normal rat the ingestion of thyroid results in the premature expulsion of a great number of reticulated cells, much the same as has been observed in pernicious anemia in man after liver therapy. In pernicious anemia it has been shown that when the marrow is crowded with megaloblasts and the process of growth is enhanced (due to liver therapy) most of the cells immediately entering the circulation will be reticulocytes (Minot and Murphy, 1928). On the other hand when many of the cells of the marrow are normoblasts, and growth is accelerated the cells enter the blood stream, not as reticulocytes, but as adult red blood cells. This fact does not explain the reticulocyte response to thyroid feeding in the cretins, inasmuch as few megaloblasts are present in the bone marrow. It is possible that a very rapid megaloblastic formation occurs as the first immediate response to the ingestion of thyroids, and in the next few days these are expelled prematurely as reticulocytes. Another possibility suggests itself in the analogy which may be drawn from the initial effect upon the bone marrow caused by ascending to high altitudes. It has been demonstrated that the increased oxygen want of such a process causes large numbers of reticulated cells to appear in the blood stream of healthy persons. It is conceivable that in the cretins the stimulus to metabolism initiated by the large amounts of thyroid suddenly bathing the tissue causes an acceleration of growth in the cells of the bone marrow, beyond the ability of the anemic animal to supply proper amounts of oxygen. This would result in a temporary oxygen want comparable to that which occurs upon ascending to high altitudes.

SUMMARY

The anemia of hypothyroidism (rabbit) is characterized by a greater than normal diameter of the erythrocytes; a color index greater than 1; a normal HCl content of the gastric contents; and a bone marrow which does not simulate either grossly or microscopically the bone marrow of pernicious anemia.

The cholesterol content of the blood, accompanying the anemia of hypothyroidism, is greater than normal.

Experimental hyperthyroidism in the rabbit causes an initial polycythemia and a corresponding increase in hemoglobin. Prolonged hyperthyroidism results in an anemia characterized by a color index less than one and a decreased diameter of the red blood cell.

The total blood volume in experimental hypo- and hyperthyroidism (dye

method) does not change beyond the limits of normal variation. In the normal adult rabbit, desiccated thyroids in dosages of 250 to 450 mgm. per day per rabbit, does not cause an appreciable increase in the percent of reticulocytes. In the cretin a reticulocyte response varying from 4 to 10 per cent occurs as the result of thyroid feeding.

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TESTICULAR DEGENERATION DUE TO INADEQUATE VITAMIN A IN CASES WHERE E IS ADEQUATE[1]

HERBERT M. EVANS

From the Institute of Experimental Biology, University of California

Received for publication July 21, 1931

Somewhat over a year ago Mason[2], to whom we are indebted for an excellent series of studies on the relation of the male gonads to various dietary regimes, reported the degeneration of the seminiferous epithelium in rats "fed purified diets adequate in all respects except for deficiency in vitamin A. Supplementing these diets with large amounts of vitamin E produced no protection against these changes." The present work, begun before the publication of Mason's results, will be regarded, we trust, as an adequate substantiation of his claim. It is presented, however, not merely because of differences in the diets and methods employed in the two researches, but primarily because of our attention to the question of adequacy of the amounts of vitamins A and E—a question unfortunately left open in the Mason work. It is certainly necessary, we believe, to provide a control group of animals fed adequate amounts of vitamin A as proof that the simultaneously administered E was adequate.

As a preliminary effort 12 animals were reared on an A-free diet[3] and 20 drops of wheat germ oil per day. A control group of 12 animals re-

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council and from the Bureau of Dairying of the United States Department of Agriculture. These funds have been generously augmented by the Board of Research and by the College of Agriculture of this University.

[2] Mason, Karl E., Journal of Experimental Zoology, 1930, lv, 101.

[3] Two diets low in vitamin A were used in this series—diets 411 and 421A. The composition of the two diets differed only in the type of casein used. Casein extracted with hot alcohol was used in diet 411 in place of the unextracted casein used in diet 421A. Animals on diet 421A exhibit symptoms of vitamin A deficiency later than those on diet 411 but in this experiment the administration of 20 drops of wheat germ oil obscured this difference and the results obtained from the two groups were essentially the same.

Diet 411		*Diet 421A*	
Casein (ext. with hot alcohol)	25.0	Casein (unextracted)	25.0
Cornstarch (cooked)	57.0	Cornstarch (cooked)	57.0
Yeast	8.0	Yeast	8.0
Lard (irradiated)	10.0	Lard (irradiated)	10.0
Salt mixture # 185	4.0	Salt mixture # 185	4.0

ceived the same diet supplemented with drops of wheat germ oil and 2 drops of cod liver oil daily. Inasmuch we can rescue any particular pregnancy in a female proven to be steri[illegible]om defective E by the simple conveyance of a single drop of wheat ge[illegible] oil daily during the period of gestation, the reader will observe that [illegible] employment of 20 drops daily in our experiments with males furnished them a very great excess of E. We realize that the amounts of vitamin A in this high dosage of wheat germ oil were not inconsiderable but previous work in the rearing of females with these high amounts of wheat germ oil (20 drops daily) had demonstrated that they ultimately exhibited the characteristic sign of A deficiency (cornified cell vaginal smears), usually at about the third month. But in order to show that the *identical* ration to which the males were submitted would produce this pathognomonic sign of A deficiency in females an equal number of females—12 litter mate sisters of the males employed—were reared on this ration. The sisters began to show A impairment (prolonged cornified cell vaginal smears) at the 90th day, though by the end of the eighth month their fertility was as yet unimpaired. The males at this time were without impairment of fertility or either customary sign of A deficiency (xerophthalmia or growth impairment). It now appeared to us as certain that the amount of A conveyed in the wheat germ oil was too much to lead us to expect A impairment in the earlier or vigorous period of the animal's life. We consequently sought to precipitate A impairment by the entire withdrawal of wheat germ oil which, we argued, would have led to extensive storage of E but surely slighter storage of A. The wheat germ oil was accordingly stopped in the case of both groups of males (those receiving cod liver oil and those without this) and in about two months the group without cod liver oil declined and at autopsy showed degeneration of the testes while the brothers from the group receiving two drops of cod liver oil daily, autopsied at the same time, showed no such testicular changes.

It might still not seem clearly proven that the testicular degeneration which followed the withdrawal of wheat germ oil in the group of males which had never received cod liver oil was due solely or chiefly to withdrawal of the A contained in the wheat germ oil. One might still consider the possibility of defective storage of E when the simultaneous inflow of A is low or defective. We regret we did not test this point directly by subsequently conveying high A to animals which had been on a low intake of vitamin A previously.

We are fortunately in the possession of facts which enable us to make a nice discrimination in the storage of vitamins A and E in growing animals. Young animals, born and nursed by mothers with normal A, store only inconsiderable amounts of it, so that if on the day of weaning they are put on an A-free regime, they begin to fail from lack of A as early as the

50th day of life. Young, born and nursed by mothers receiving normal amounts of E, store very appreciable amounts of the fertility vitamin, so that when placed on the day of weaning on an E-free regime, they remain fertile for some time. Males, for instance, regularly retain a normal seminiferous epithelium and fertility until the 90th day of life.[4]

These facts, ascertained repeatedly, establish the earlier appearance of A delinquincy—its appearance at a time when the body stores of E are demonstrably adequate. If, in this early failure due to A, the testicular epithelium is still intact, we would be justified in regarding its final destruction as due to low E, but if, on the other hand it is already critically impaired at a time when E is known to be adequate, it would seem that we could justifiably regard its destruction as due to low A.

Accordingly a group of 24 animals whose mothers had been transferred to diet 411 and 20 drops of wheat germ oil daily on the 15th day of lactation was divided in the following manner: eight animals were fed diet 411 plus six drops of wheat germ oil daily, eight animals diet 411, and the remaining eight diet 411 plus two drops of cod liver oil daily. The growth (fig. 1) of the animals was studied and after the group receiving unsupplemented diet 411 had appreciably declined in weight the entire group was autopsied. At this time seven of the eight animals on diet 411 had xerophthalmia, while those on diet 411 and six drops of wheat germ oil showed no eye symptoms, and all but three of the group (GH7822, W7805, BH7819) were gaining weight. At autopsy one testis was fixed for histological studies and the weight of the second was determined. The testes of the animals on diet 411 were not only smaller, as we would expect, but the ratio of the weight of the testis to the body weight was lower. The complete data for the series are found in table 1.

The fresh fluid from the epididymis was studied in each case in order to determine the type and motility of sperm present, or the absence of sperm as was found in the case of all but one (W7800) of the animals receiving diet 411. The fluid from the epididymis of those animals receiving the six drops wheat germ oil contained non-motile sperm in all but two cases. One of these animals (BH7811) exhibited more marked changes, i.e., the complete absence of sperm while the second (W7799) was not so seriously affected and exhibited motile sperm. The group of animals with the supplement of cod liver oil had motile sperm in all cases.

The histological examination of the testes revealed changes which paralleled those obtained by the study of the fluid from the epididymis. The

[4] This unavoidable initial fertility of animals reared on an E free regime at one time constituted an embarrassment in our studies, and has doubtless even prevented some investigators from recognizing the existence of dietary sterility due to the lack of the specific vitamin E. See chapter 3, The Antisterility Vitamine Fat Soluble E—Evans, H. M. and Burr, G. O. Memoirs of the University of California, Vol. 8.

TABLE 1

Showing testicular degeneration in young (60–72 day old) rats due to low A in the period when their initial stores of E are adequate

DESIGNATION OF ANIMAL	AGE AT AUTOPSY	INCIDENCE OF XEROPHTHALMIA	BODY WEIGHT AT AUTOPSY	WEIGHT OF ONE TESTIS	WEIGHT OF TESTIS PER 100 GRAMS OF BODY WEIGHT	OBSERVATION OF FLUID FROM EPIDIDYMIS	HISTOLOGICAL FINDINGS IN THE TESTIS
Diet 411 (Devoid of conveyed A or E)							
	days		*grams*	*grams*	*grams*		
B7809	72	–	120	0.40	0.33	No sperm	
BH7806	72	+	96	0.25	0.26	No sperm	
W7800	60	+	138	0.60	0.43	Some motile sperm; others non-motile	Partial degeneration. Some tubules still have spermatozoa many of which look abnormal. Most of tubules definitely degenerated though all still possess some cells
GH7797	64	+	100	0.60	0.60	No sperm	Every tubule greatly degenerated. (A few cells left)
W7791	65	+	96	0.20	0.20	No sperm	
GH7817	64	+	88	0.25	0.28	No sperm	
G7820	64	+	66	0.16	0.24	No sperm	Every tubule greatly degenerated. (A few cells left)
B7788	65	+	118	0.45	0.38	No sperm	
Average.....			103	0.36	0.34		
Diet 411 and 6 drops wheat germ oil daily (High E low A)							
GH7822	71	–	96	0.45	0.46	Non-motile sperm	A few tubules look normal—others partially degenerated
BH7811	72	–	79	0.20	0.25	No sperm	
W7799	72	–	160	0.65	0.40	Motile sperm	Some degeneration—a few tubules looked normal, many have fair number of cells left, a few are almost completely gone
B7808	72	–	120	0.35	0.29	Only a few non-motile sperm	
W7790	72	–	182	0.60	0.33	Fused sperm	All tubules partially degenerated

TABLE 1—*Concluded*

DESIGNATION OF ANIMAL	AGE AT AUTOPSY	INCIDENCE OF XEROPHTHALMIA	BODY WEIGHT AT AUTOPSY	WEIGHT OF ONE TESTIS	WEIGHT OF TESTIS PER 100 GRAMS OF BODY WEIGHT	OBSERVATION OF FLUID FROM EPIDIDYMIS	HISTOLOGICAL FINDINGS IN THE TESTIS
Diet 411 and 6 drops wheat germ oil daily (High E low A)—*Concluded*							
	days		*grams*	*grams*	*grams*		
GH7796	71	—	150	0.90	0.60	Few motile sperm; others non-motile	About ½ tubules look normal, others partially and a few completely degenerated
W7805	72	—	108	0.50	0.46	Few feebly motile sperm; others non-motile	
BH7819	71	—	138	0.70	0.51	Few non-motile sperm	
Average			129	0.54	0.41		
Diet 411 and 2 drops cod liver oil daily (High A, no conveyed E)							
BH7807	72	—	126	0.90	0.71	Motile sperm	
BH7818	71	—	136	1.10	0.81	Motile sperm	Normal
G7795	72	—	206	1.30	0.63	Motile sperm	Normal
BH7810	72	—	184	1.50	0.82	Motile sperm	
W7804	72	—	140	1.20	0.85	Motile sperm	
B7789	72	—	200	1.10	0.55	Motile sperm	Normal
GH7798	72	—	232	1.40	0.60	Motile sperm	
GH7821	71	—	126	0.70	0.55	Motile sperm	Partial degeneration
Average			168	1.15	0.69		

testes from the group of animals receiving diet 411 showed marked degeneration, practically every tubule being involved. In a few of the tubules the germinal epithelium had completely disappeared leaving only a network of fibers in which a few Sertoli nuclei were held. The majority of the tubules retained some of the elements of the germinal epithelium. Spermatogonia and spermatocytes could be seen in many tubules but spermatozoa were rarely found.

The animals receiving the supplement of six drops of wheat germ oil possessed testes which showed some degeneration, that is, some tubules were normal and others partially degenerated. The groups of animals receiving two drops cod liver oil had normal testes in all cases.

The above series of animals fortify the finding that in growing males

the stores of vitamin A are more rapidly depleted than those of vitamin E. The animals not only showed the signs characteristic of vitamin A

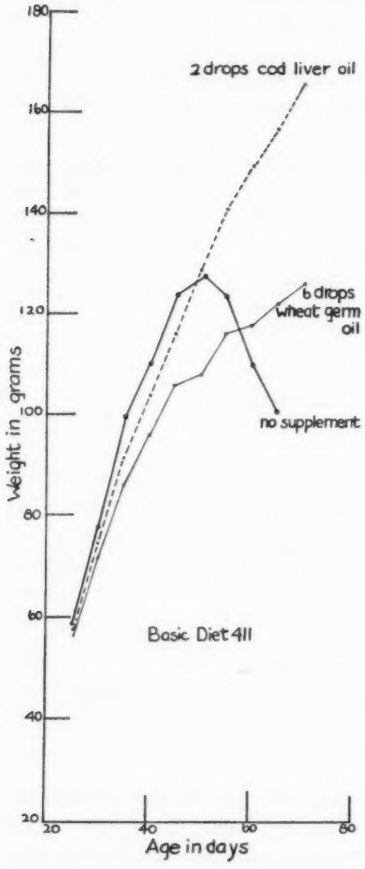

Fig. 1

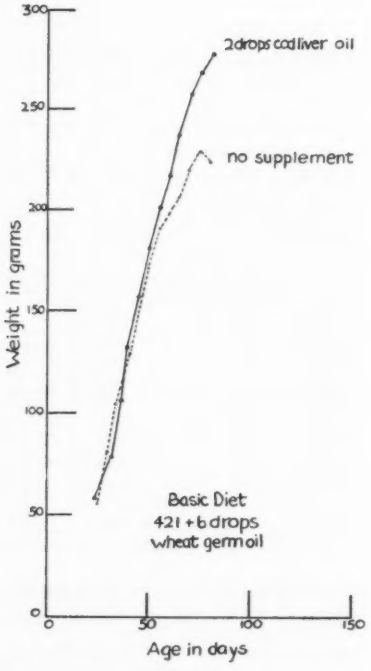

Fig. 2

Fig. 1. Each curve represents the average growth of eight male rats on diet 411 with no added vitamin A or E, with high vitamin E and low A and with low vitamin E and high A.

Fig. 2. Each curve represents the average growth of ten males on diet 421 supplemented with high vitamin E and low A in one case, and high vitamin E and high A in the second case.

deficiency—decline in weight and xerophthalmia—but the litter-mate sisters studied at the same time showed continuous cornified cells in the

vagina. The experiment seems to show conclusively that in the presence of vitamin E stored from the lactation period and with the depletion of the vitamin A stored, there is marked deterioration of the seminiferous epithelium.

The above experiments involved two extremely different situations: 1, a considerable amount of vitamin A was fed and many months passed before the animals showed decline due to low A, in fact, the decline set in only after withdrawal of A which also involved the vitamin E, and there thus entered the problem of vitamin storage of which little is known in this epoch of the animal's life: 2, the stores of vitamin A were so rapidly depleted that the animals succumbed to the deficiency before the age of sexual maturity was reached.

Accordingly, a new series of experiments was devised. Twenty males were taken at the 21st day of life and divided into two groups of 10 animals each. They were reared in individual cages on diet 421[5] and six drops of wheat germ oil daily. One group received two drops of cod liver oil daily, the other being without cod liver oil and therefore dependent for its vitamine A intake solely on the amount conveyed by the wheat germ oil. The average growth obtained in the two groups is illustrated in fig. 2.

The sexual activity of each animal was tested, beginning at the 50th day of life, by introducing females at estrus into the cage of the male, with examination the following morning for plug and sperm. The female was studied to determine whether a litter resulted. The advent of sterility in the male was detected by the failure of known copulations to result in pregnancy in females of proven fertility.

The inception of sterility in the group of animals reared on diet 421 and six drops wheat germ oil varied from 74 to 117 days (average 89 days). At autopsy the fresh fluid from the epididymis was studied and either abnormal sperm or no sperm were found. Histological examination of the testes revealed degeneration of the seminiferous epithelium in all cases examined. The data for the group are listed in table 2.

[5] The composition of diet 421 is essentially the same as diet 411 with the exceptions of the casein which is unextracted and the lard which is slightly rancid. The rancid lard is added in order to destroy any A present in the constituents of the diet. The animals receiving diet 421A supplemented with 2 drops of cod liver oil thrive very well on this diet, indicating that there are no toxic effects from the lard.

Diet 421

Casein (unextracted)	25.0
Cornstarch (cooked)	57.0
Yeast	8.0
Lard (slightly rancid) irradiated	10.0
Salt mixture # 185	4.0

TABLE 2

Showing testicular degeneration in rats due to low A at the inception of sexual maturity

Diet 421 with 6 drops wheat germ oil daily

DESIGNATION OF ANIMAL	NUMBER OF INSTANCES IN WHICH AN ESTROUS FEMALE WAS INTRODUCED INTO MALE'S CAGE	NUMBER OF COPULATIONS	NUMBER OF LITTERS SIRED	LAST FERTILE MATING (AGE IN DAYS)	FIRST STERILE MATING (AGE IN DAYS)	AUTOPSY (AGE IN DAYS)	INCIDENCE OF XEROPHTHALMIA	BODY WEIGHT AT AUTOPSY	WEIGHT OF ONE TESTIS	WEIGHT OF TESTIS PER 100 GRAMS BODY WEIGHT	OBSERVATION OF FLUID FROM EPIDIDYMIS	HISTOLOGICAL FINDINGS IN THE TESTIS
								grams	*grams*	*grams*		
BH6594	4	2	2	82	None	90	+	228	1.20	0.52	Motile sperm	
W6617	3	1	1	74	None	80	−	200	1.10	0.55	Motile sperm	Tubules appear normal
GH6628	2	2	2	82	None	87	+	214	1.32	0.61		
W6634	7	6	3	85	106	159	+	286*	0.55	0.19	A few non-motile sperm	Marked degeneration; no normal tubules
W6666	10	5	3	94	105	159	−	224	0.90	0.40	Non-motile fused sperm	Marked degeneration; no normal tubules
GH6585	11	7	4	117	154	160	−	296**	1.15	0.38	No sperm	Most tubules completely degenerated; a few normal
BH4314	9	3	2	92	None	112	+	172	0.47	0.27	Motile sperm	
BH6636	3	0	0	0	None	Died at 74 days						
G6582	10	5	3	88	112	Died at 127 days***						
W6584	Died at 67 days											

* At 95 days of age W6634 began to lose weight rapidly. Two drops of cod liver oil were fed on 2 successive days, growth was rapidly resumed.

** GH6585 was losing weight at 91 days of age—Two drops of cod liver oil were fed on 3 successive days—there was immediate resumption of growth.

*** G6582 was losing weight at 94 days of age and was given 2 drops of cod liver oil on two successive days—growth was resumed at once.

The group of animals receiving cod liver oil was tested in the same manner as their littermate brothers. The fertility of the male was not impaired and at autopsy the findings indicated normal conditions. The complete data can be found in table 3.

These experiments seem to demonstrate clearly that the destruction of the seminiferous epithelium in the animals receiving six drops of wheat germ oil daily but without cod liver oil, was due to a deficient intake of vitamin A. That the E in six drops of wheat germ oil was adequate would appear to have been conclusively shown by the retention of normality in all animals of the group receiving the same amount of wheat

TABLE 3

Showing testicular normality when the diet is identical with that employed in table 2 but with addition of cod liver oil

Diet 421 with 6 drops wheat germ oil and 2 drops cod liver oil daily

DESIGNATION OF ANIMAL	NUMBER OF INSTANCES IN WHICH AN ESTROUS FEMALE WAS INTRODUCED INTO MALE'S CAGE	NUMBER OF COPULATIONS	NUMBER OF LITTERS SIRED	AGE AT TERMINATION OF EXPERIMENT	BODY WEIGHT AT TERMINATION OF EXPERIMENT	WEIGHT OF 1 TESTIS	OBSERVATIONS OF FLUID FROM EPIDIDYMIS	HISTOLOGICAL FINDINGS IN THE TESTIS
				days	*grams*	*grams*		
B6593	8	3	3	161	360			
B6592	6	4	4	161	412			
GH6583	8	6	6	161	372			
BH6667	12	4	4	159	363	1.75	Motile sperm	Normal
W6635	7	6	6	160	406	1.60	Motile sperm	Normal
GH6629	5	5	5	161	385			
W6618	5	5	5	160	354	1.65	Motile sperm	Normal
W6665	10	3	3	161	358			
GH4315	6	5	5	160	364			
BH6586	9	5	4	160	420	1.60	Motile sperm	Normal

germ oil but with two drops of cod liver oil, a substance known to contain no E.

That the decline of the first group of animals was due to deficient A was shown by: 1, the characteristic signs of A deficiency appearing in some of them (xerophthalmia in four instances out of ten) and appearing in all cases in a group of sister animals receiving the same ration (continuous cornified cell vaginal smear at the beginning of the 50th day of life); 2, when rapid decline in weight began, a few drops of cod liver oil led to immediate resumption of growth, which lasted from twenty to sixty days (GH6634, G6582).

SUMMARY

1. The vitamin A stored during the intra-uterine and suckling life of the rat is more rapidly depleted than the vitamin E.

2. Males maintained on diets adequate in vitamin E and low in vitamin A (the amount contained in 6 drops wheat germ oil) become sterile by the third month of life, though when entirely without E and with adequate A, sterility does not supervene until later.

3. Examination of the fresh fluid from the epididymis and histological examination of the testes indicate that marked degeneration of the testes had taken place in the absence of vitamin A and in the presence of adequate vitamin E.

STUDIES IN THE PHYSIOLOGY OF EXERCISE

VIII. The Chronic Effects of Running and Swimming on the Hearts of Growing Dogs as Revealed by Roentgenography[1]

ARTHUR H STEINHAUS, JOHN P. KIRMIZ and KNUD LAURITSEN

From The Young Men's Christian Association College Laboratory for Physiologic Research in Physical Education, Chicago

Received for publication September 9, 1931

In this paper are reported roentgenographic observations on growing dogs some of which were caused to exercise while littermates were held as unexercised controls. Further papers will report electrocardiographic and post-mortem organ-weight findings on the same dogs. The data here presented support the view that exercise accelerates the growth of the heart. In the face of an already large literature our paper deserves attention for presenting *growth curves* of the heart constructed from measurements of roentgenograms taken biweekly throughout the period of growth with the effects of exercise reflected in modifications of this growth curve. Grober (1908) and Thörner (1930) each observed the size of heart shadows in dogs before and after a period of training. Hiramatsu (1919) made similar observations on rats. Their observations support the findings of the larger group of workers whose studies were confined to autopsy material, namely, that exercise induces cardiac hypertrophy.

The studies on man have led to conflicting results. Since Deutsch and Kauf (1927), Rautmann (1927) and Podkaminsky (1929) have each reviewed the literature only a very brief statement is in place here. Two factors contribute to the indecisive nature of the published findings on man. In the first place the shadow of the heart in man fuses at its lower border with that of the diaphragm, and secondly, conclusions have been drawn from comparisons of the findings in athletes with standards or norms of which there are a large variety. Some who recognize an enlarged shadow say it is due to a greater relaxation and consequent diastolic filling due to the vagotonic slowing of the heart (Deutsch and Kauf, 1927). Others who find this enlarged outline only in athletes trained for endurance events account for it as frank hypertrophy which, however, may regress upon the cessation of training (Herxheimer, 1929). A third group

[1] A grant from the Carnegie Corporation of New York has aided the conduct of this study.

insists that when the correct normal figures are employed there is indication of hypertrophy only in exceptional cases and then it should be viewed as not certainly harmless (Rautmann, 1927). Only two workers have published "before and after" studies on man. Ackermann (1927) from orthodiagrams on a group of oarsmen secured before and after a season of training concludes that in most novices and in some veterans a period of strenuous training induces cardiac hypertrophy. Purjesz et al., (1930) in a similarly controlled study find slight increases in the transverse due to a period of training in running and swimming.

EXPERIMENTAL METHODS. In the course of this experiment the male puppies of seven different litters were caged as soon as they were weaned. Losses at very early ages required the disposition of three complete litters. The ranks of two others were thinned by an attack of the pulmonary form of distemper which afflicted also the survivors in litters D, E, and H. In all, four litters consisting of three dogs each, survived to furnish data for this experiment. Each of the dogs which succumbed to distemper was a control of a litter in which we had, fortunately, held two as controls.

Exercise. In each of the four litters consisting of three dogs one was exercised by running, a second by swimming, while a third served as unexercised control. All of the dogs were permitted to play for a brief period twice a day in an open yard. On top of this basic activity the runners were daily placed into a free running treadwheel about six feet in diameter constructed of wood with a hard maple floor and screened sides. The wheel is wide enough to permit three dogs to run side by side. We found it of great advantage in teaching a new dog or encouraging a phlegmatic one, to put him into the wheel with an experienced, good runner. The distance thus voluntarily run was estimated from the readings of an attached cyclometer—297 revolutions constituting a mile. A cement tank with water at 25°C. was used for swimming. As the dogs developed skill they were loaded by fastening weights about their shoulders. Each dog was swam daily at least once until he showed signs of fatigue. The time swam and weight of load were recorded.

Food. As soon as the puppies were old enough they were placed on the standard laboratory diet consisting of a mixture of oatmeal, Swift's Silver Fur Food, powdered skimmed milk, cod liver oil, and salt. Daily between 6 and 10 p.m. they were permitted to eat all they desired. Young dogs were fed oftener. This diet was supplemented daily by a small bone and in the latter half of the experiment, twice weekly by a generous supply of ground beef lung. The animals were weighed twice weekly.

Records. The reliability of radiography in preference to orthodiagraphy has repeatedly been attested for human work (Bardeen, 1917; Köhler, 1928) but especially for animal work it is unquestionably favored (Skavlem, 1922; Eyster, 1928). Though we realized the greater accuracy of the

standard 2m. teleroentgenographic distance our equipment at the beginning of these experiments did not permit for a distance greater than 89 cm. between target and film. We now have in progress further studies in which a six foot distance is employed. Our learnings from a comparison of the two distances are discussed below. The exposure time was two seconds. The kilovoltage and milliamperes were varied as the requirements for securing clear outlines changed in the growing dogs. Two men held the animal in supine position, his back resting on the cassette fitted with Patterson screens. Every effort was made to keep the head in straight line with the spine and the entire body balanced with sternum and vertebral column in the same vertical plane. After a few seconds of calm in this position, exposure was made beginning at the height of inspiration. Duplicate exposures were made and immediately processed and checked for workableness. When necesary, further exposures were made until two clear pictures were secured. The film was then placed against an illuminated frosted window and the cardiac shadow carefully outlined with a red pencil. To insure uniformity of technique all of the outlining was done by the same individual. In most cases no difficulty was experienced in outlining the heart silhouette, clearly and accurately. Since in the dog, unlike the condition in man, the heart is free from all diaphragmatic interference in the chest cavity, the only place where an error of judgment might be introduced is in tracing the contour of the base of the heart. Since this represents only a small fraction of the heart outline it becomes simple, with a little experience to complete the outline in a consistent fashion by joining the upper left and right borders in a curve. Four measurements were then taken from each silhouette. The area was measured with a polar planimeter. This was done by tracing the circumference once in the beginning, five consecutive times subsequently, and then taking the average of the six rounds as the reading. The other three dimensions taken were the greatest length, the greatest breadth at right angles to it, and the transverse. For these purposes a millimeter squared paper was devised. By placing the film against it and both against the illuminated window the desired dimensions could be read off easily with an accuracy of half a millimeter. The values for area, length, breadth, and transverse measured from each of the duplicate exposures were then averaged and this figure recorded as final for the period. Heart volume was calculated from area by the formula, Vol. $= 0.44A^{3/2}$ (Skavlem, 1922). This volume in cubic centimeters divided by the dog's weight in kilograms gave us the volume to weight ratio $\left(\frac{\text{Vol}}{\text{Wt}}\right)$. The greatest length divided by the greatest breadth at right angles to it is recorded as the length to breadth ratio $\left(\frac{\text{L}}{\text{B}}\right)$. The data accumulated in the manner here described over suitably long periods

before, during and in some cases after exercise were used in the preparation of the accompanying tables, graphs and figures.

RESULTS. Each dog in this experimental series is designated by a number which applies specifically to him and by a letter which he shares with his littermates. In this paper we are reporting results secured on dogs 25B, 26B, 27B, 34D, 36D, 37D, 38E, 39E, 40E, 46H, 47H, and 49H. The data find expression in three forms: 1. Tables for each litter giving the age in days, weight, heart area, volume to weight ratio $\left(\frac{\text{Vol}}{\text{Wt}}\right)$ and length over breadth ratio $\left(\frac{\text{L}}{\text{B}}\right)$. (The transverse dimension which was also measured has been discarded in the interest of brevity since no particular significance attaches to it in the dog.) 2. Graphs showing *a*, the growth curve of the heart area, and *b*, $\frac{\text{Vol}}{\text{Wt}}$ curves which may be read as cubic centimeters of heart per kilogram of dog. 3. Outlines representing the cardiac silhouette traced directly from the original film taken at various times before and after exercise. Tables 1 to 4 report findings on litters B, D, E, and H consecutively. The graphs for litters B, D, E, and H have been combined in figure 1. To conserve space the outlines of cardiac silhouettes are reproduced for only one litter (fig. 2). These are essentially representative of this type of data for our entire series. The details of results are best presented by litters.

Litter B is composed of dogs 25, 26, and 27 of unknown stock. Table 1 and figure 1 give the results obtained. The cardiac areas indicate that in the beginning the swimmer, dog 27, had the smallest heart, and the runner, dog 26, the largest. Immediately before exercising the heart of the control was just under that of the swimmer. Exercise was begun on the 242nd day. In the following 65 days a total of 32 hours was spent in trying to teach dog 26 to run. During this period he ran very little. No other dog learned so slowly. His heart does not deviate from that of the control. After a two week rest, and beginning with the 328th day, more success was experienced in getting him to run. From then on to the 561st day when exercise was discontinued his cardiac size is consistently larger than that of the control. This is even more noticeable in the volume over weight curve. In this second period of exercise he ran a distance of 1400 miles in 233 days averaging about 6 miles per day. With the remission of exercise both curves reflect a prompt reduction in heart size. On the 636th day exercise was resumed and continued until the 657th day. In these 21 days he ran 130.4 miles and the heart curves show first a marked drop and then a slight rise. In the ninety-four days following the beginning of exercise on the 242nd day, dog 27 swam 48 hours without weights. His heart begins almost immediately to show marked enlargement. From

TABLE 1

Roentgenogram data from litter B

AGE	DOG 25 CONTROL				DOG 26 RUNNER				DOG 27 SWIMMER			
	Weight	Area	Vol. / Wt.	L / B	Weight	Area	Vol. / Wt.	L / B	Weight	Area	Vol. / Wt.	L / B
days	*kgm.*	*sq. cm.*			*kgm.*	*sq. cm.*			*kgm.*	*sq. cm.*		
105	5.3	33.7	16.1	1.31								
129	6.8	38.5	15.4	1.29	6.8	35.2	13.5	1.49	6.8	29.5	10.3	1.37
141					8.3	38.8	12.8	1.41	7.5	35.2	12.2	1.38
149	9.0	42.0	13.3	1.40	8.9	37.5	11.3	1.36	8.4	37.9	12.1	1.45
167	9.8	45.7	13.8	1.31	9.5	43.7	13.4	1.34	10.8	44.5	12.1	1.36
174	10.6	45.4	12.6	1.28	10.2	46.8	13.7	1.34	11.0	45.7	12.3	1.36
188	11.0	46.0	12.5	1.33	10.6	50.8	13.0	1.48	11.7	47.4	12.2	1.40
201	11.3	53.7	15.3	1.24	11.3	51.5	14.5	1.32	12.9	54.4	13.6	1.33
217	12.1	50.1	12.9	1.31	12.2	54.2	14.4	1.36	13.6	57.9	14.2	1.28
231	12.7	53.4	13.5	1.36	12.9	61.5	16.5	1.39				
					Began running on 242nd day				Began swimming on 242nd day			
252	13.8	55.9	13.3	1.39	13.7	62.1	15.7	1.35	15.7	61.8	13.6	1.29
266	13.8	58.8	14.2	1.36	13.6	58.5	14.4	1.41	15.9	63.4	14.0	1.33
282	14.5	61.5	14.6	1.35	14.2	62.3	15.2	1.38	16.7	64.9	13.8	1.30
296	14.6	66.3	16.2	1.30	14.2	65.2	16.3	1.42	16.7	69.7	15.3	1.34
311	14.8	63.0	14.8	1.32	14.5	63.0	15.1	1.41	16.9	71.6	15.8	1.38
321	14.2	59.7	14.3	1.45	13.4	63.1	16.4	1.44	16.2	65.8	14.5	1.44
					Began to run well on 328th day							
335	13.8	61.6	15.4	1.34	12.9	62.6	16.9	1.48	15.6	63.9	14.4	1.46
350	14.2	59.3	14.2	1.42	13.0	63.8	17.3	1.48	16.1	73.2	17.1	1.40
365	14.7	63.0	14.9	1.31	13.5	61.8	15.8	1.53	16.7	75.1	17.1	1.38
378	15.1	66.7	15.9	1.34	13.5	69.8	19.0	1.43	17.1	76.8	17.3	1.42
393	15.4	67.8	15.9	1.35	13.9	69.7	18.4	1.54	17.3	77.1	17.2	1.43
425	15.4	65.9	15.3	1.36	13.9	71.9	19.3	1.45	Accidental death			
437	15.7	66.1	15.1	1.41	14.2	72.2	19.0	1.48				
451	15.6	66.4	15.2	1.35	14.4	74.5	19.6	1.43				
465	16.0	68.3	15.5	1.38	14.1	74.7	20.1	1.36				
479	16.5	70.1	15.6	1.30	14.4	74.1	19.4	1.42				
493	16.0	67.1	15.1	1.40	14.7	73.0	18.7	1.36				
504	16.2	69.7	15.8	1.38	14.6	73.4	18.9	1.43				
522	16.0	66.0	14.8	1.34	14.7	73.7	18.9	1.45				
532	15.8	66.5	15.1	1.31	14.7	78.1	20.6	1.42				
546	15.9	68.4	15.6	1.32	14.5	80.1	21.7	1.48				
560	16.5	66.9	14.6	1.32	14.6	81.1	21.9	1.42				
					Suspended running 561st day							
574	16.8	66.6	14.2	1.33	14.7	78.5	20.7	1.44				
588	16.9	67.3	14.4	1.35	15.1	79.4	20.6	1.42				
602	16.6	71.0	15.8	1.33	15.0	78.3	20.2	1.48				
616	17.2	67.2	14.1	1.30	15.4	77.0	19.2	1.42				
630	18.1	66.6	13.2	1.32	15.7	78.1	19.3	1.42				
					Resumed running 636th day							
644	19.7	67.2	12.3	1.40	15.6	74.8	18.2	1.40				
656	19.7	69.5	12.9	1.32	15.6	76.1	18.7	1.40				

the 336th to the 399th day he swam 22½ hours with an average weight of 3 kgm. Unfortunately on the 402nd day he was lost to the series, due to

TABLE 2

Roentgenogram data from litter D

AGE	DOG 36 CONTROL				DOG 34 RUNNER				DOG 37 SWIMMER			
	Weight	Area	Vol./Wt.	L/B	Weight	Area	Vol./Wt.	L/B	Weight	Area	Vol./Wt.	L/B
days	*kgm.*	*sq. cm.*			*kgm.*	*sq. cm.*			*kgm.*	*sq. cm.*		
68	4.3	28.7	15.7	1.16	3.5	21.9	12.9	1.35	4.3	28.1	15.1	1.12
84	5.4	33.7	14.9	1.18					5.4	28.8	12.6	1.21
100	6.5	38.2	15.9	1.36	5.7	34.4	15.5	1.33	6.7	36.6	14.8	1.36
113	7.5	41.6	15.7	1.29	6.5	34.5	14.0	1.51	8.0	39.5	13.5	1.21
131	7.7	43.0	16.1	1.38	6.7	36.7	14.6	1.43	7.8	40.2	14.4	1.33
					Began running 142nd day				Began swimming 142nd day			
146	7.9	46.1	16.1	1.47	6.8	36.7	14.4	1.40	8.2	42.1	14.6	1.46
161	10.0	53.1	17.0	1.40	9.0	47.1	15.7	1.38	10.1	57.0	18.7	1.40
174	11.0	58.0	17.6	1.38	9.8	47.7	14.7	1.47	11.4	58.6	17.2	1.48
189	12.6	59.7	16.1	1.35	11.0	54.5	16.1	1.42	12.8	68.1	19.3	1.38
221	12.5	63.9	18.0	1.40	11.0	55.9	16.7	1.44	12.2	69.4	20.8	1.52
					Suspended running 215th day				Suspended swimming 215th day			
233	13.7	66.0	17.2	1.45	11.6	59.7	17.5	1.41	13.3	71.2	19.8	1.41
247	13.6	68.6	18.3	1.52	11.4	51.1	14.5	1.33	13.1	66.6	18.3	1.55
									Resumed swimming 256th day			
261	15.2	79.2	20.4	1.44	12.4	59.2	16.1	1.41	14.6	70.5	17.8	1.43
					Resumed running 265th day							
275	16.2	79.2	19.1	1.38	13.7	63.7	16.4	1.37	16.1	78.5	19.0	1.34
289	17.2	79.5	18.1	1.35	14.7	65.1	15.7	1.39	17.5	79.9	17.9	1.34
303	17.6	80.6	18.0	1.36	15.1	68.7	16.6	1.37	17.8	81.9	18.3	1.44
321	18.2	84.5	18.7	1.41	15.4	66.5	15.5	1.39	18.2	86.7	19.5	1.38
331	18.5	84.1	18.3	1.45	15.5	68.3	16.0	1.38	18.4	85.6	18.9	1.41
345	18.7	86.5	18.9	1.41	15.8	71.0	16.6	1.37	18.8	87.1	19.0	1.41
359	19.3	86.4	18.3	1.37	16.5	72.6	16.5	1.37	18.9	87.7	19.0	1.35
373	19.4	87.9	18.7	1.35	16.5	71.0	15.9	1.43	19.1	86.2	18.4	1.41
387	19.3	91.0	19.8	1.39	16.5	73.0	16.6	1.38	18.9	89.0	19.5	1.45
401	19.3	89.6	19.3	1.40	16.5	74.0	17.0	1.40	18.5	88.8	19.9	1.48
415	19.8	91.2	19.3	1.34	17.2	73.4	16.0	1.35	18.9	91.6	20.4	1.41
429	20.0	93.7	19.9	1.42	17.5	72.9	15.6	1.33	19.4	91.0	19.7	1.41
443	20.2	86.0	17.4	1.39	17.8	72.1	15.1	1.33	19.8	90.5	19.1	1.34
457	20.7	89.7	18.0	1.44	17.5	64.5	13.0	1.33	20.3	86.4	17.4	1.43
467	20.7	94.3	19.5	1.41	17.5	71.3	15.1	1.35	20.3	89.0	18.2	1.43

accident. With the exception of dog 27 who at a very early age suffered a mild attack of what appeared to be the nervous form of distemper, the dogs of this litter enjoyed perfect health throughout.

Litter D comprises dogs 34, 36, and 37 of airedale and police stock. Table 2 and figure 1 give the results obtained. On the 68th day of their age, when their cardiac size was first photographed the control had the

TABLE 3

Roentgenogram data from litter E

AGE	DOG 39 CONTROL				DOG 40 RUNNER				DOG 38 SWIMMER			
	Weight	Area	Vol./Wt.	L/B	Weight	Area	Vol./Wt.	L/B	Weight	Area	Vol./Wt.	L/B
days	*kgm.*	*sq. cm.*			*kgm.*	*sq. cm.*			*kgm.*	*sq. cm.*		
61	3.4	17.2	9.3	1.26	3.9	21.8	11.3	1.11	2.7	14.2	8.7	1.32
75	4.2	18.1	8.0	1.17	4.8	23.0	10.1	1.19	3.3	17.4	9.8	1.11
90	4.7	22.8	10.1	1.31	5.7	27.4	11.0	1.22	3.7	17.8	8.9	1.22
100	4.6	24.8	11.7	1.31	5.7	27.7	11.1	1.14	3.6	19.9	10.9	1.26
114	4.8	26.6	12.5	1.31	5.8	30.3	12.6	1.27	3.6	21.0	11.7	1.26
					Began running 121st day				Began swimming 121st day			
129	5.4	29.5	13.0	1.34	6.2	31.2	12.2	1.32	3.9	23.4	12.8	1.26
144	6.0	33.2	14.0	1.37	6.6	36.1	14.5	1.35	4.4	28.1	14.8	1.31
157	6.7	34.3	13.1	1.40	6.9	41.9	17.2	1.37	4.7	29.0	14.6	1.28
172	7.2	36.8	13.6	1.34	6.9	44.2	18.7	1.33	5.0	31.2	15.2	1.21
204	6.9	37.2	14.4	1.30	7.3	45.7	18.5	1.34	4.7	31.7	16.6	1.22
									Suspended swimming 206th day			
216	7.1	38.6	14.8	1.33	7.9	48.2	18.6	1.33	5.0	32.6	16.3	1.22
230	7.3	39.9	15.1	1.40	8.0	49.7	19.2	1.34	5.3	34.1	16.5	1.22
									Resumed swimming 239th day			
244	8.0	41.8	14.9	1.39	8.0	54.4	20.5	1.32	6.0	35.7	15.6	1.31
258	8.3	42.9	14.9	1.31	8.9	55.0	20.1	1.32	6.1	37.1	16.2	1.22
272	8.5	43.5	14.9	1.29	9.7	56.6	19.3	1.23	6.1	36.5	15.9	1.25
286	8.8	44.1	14.6	1.32	10.0	59.9	20.4	1.30	6.3	38.7	16.8	1.20
304	8.7	39.0	12.3	1.33	10.1	57.7	19.0	1.31	5.4	33.6	15.8	1.14
314	8.8	44.0	14.6	1.30	10.2	60.3	20.2	1.27	5.8	33.8	14.8	1.27
328	9.2	45.8	14.8	1.34	10.1	61.6	21.1	1.32	6.1	35.0	14.9	1.21
342	9.1	45.9	15.0	1.35	10.4	60.5	20.0	1.32	6.5	38.3	16.0	1.27
356	9.2	46.5	15.1	1.21	10.5	59.0	19.0	1.30	6.5	39.0	16.5	1.21
					Suspended running 360th day							
370	9.1	45.7	14.8	1.34	10.6	60.1	19.4	1.27	6.4	38.6	16.4	1.23
384	9.2	47.2	15.5	1.30	10.8	60.2	19.1	1.30	6.4	40.0	17.4	1.28
398	9.6	46.6	14.6	1.29	11.3	61.0	18.5	1.26	6.5	40.5	17.5	1.27
412	9.5	48.8	15.7	1.31	11.4	63.5	19.7	1.23	6.6	41.4	17.7	1.26
					Resumed running 415th day							
426	9.3	49.2	16.3	1.27	11.4	60.3	18.1	1.26	6.6	39.7	16.7	1.22
440	9.5	50.8	16.7	1.26	11.6	55.4	15.6	1.27	6.6	41.2	17.6	1.16
456	9.3	48.1	15.8	1.29	11.2	58.6	17.6	1.25	6.6	39.8	16.7	1.23

TABLE 4

Roentgenogram data from litter H

AGE	DOG 46 CONTROL				DOG 49 RUNNER				DOG 47 SWIMMER			
	Weight	Area	Vol./Wt.	L/B	Weight	Area	Vol./Wt.	L/B	Weight	Area	Vol./Wt.	L/B
days	*kgm.*	*sq. cm.*			*kgm.*	*sq. cm.*			*kgm.*	*sq. cm.*		
45	3.3	19.3	11.4	1.25	3.0	18.5	11.7	1.41	3.3	21.7	13.5	1.39
59	3.7	24.8	14.5	1.29	3.3	18.6	10.7	1.48	3.9	23.1	12.5	1.47
74	4.9	28.7	13.8	1.27	4.2	23.5	11.9	1.41	4.9	25.8	11.7	1.38
89	6.7	36.6	14.5	1.38	5.7	30.6	13.0	1.40	6.7	36.4	14.4	1.34
					Began running 90th day				Began swimming 96th day			
102	7.3	39.5	14.9	1.28	6.7	33.6	12.8	1.38	8.0	41.8	14.9	1.39
117	9.1	42.9	13.6	1.38	8.1	39.5	13.4	1.48	9.7	56.2	19.1	1.33
					Suspended running 143rd day				Suspended swimming 143rd day			
149	10.4	42.8	11.8	1.51	9.3	45.5	14.4	1.59	11.1	67.1	21.8	1.33
161	11.0	50.8	14.4	1.39	9.3	47.4	15.4	1.57	11.9	69.4	21.3	1.43
					Resumed running 164th day							
175	11.0	45.4	12.2	1.48	9.5	50.6	16.6	1.57	12.5	70.6	20.9	1.43
									Resumed swimming 183rd day			
189	11.5	55.4	15.7	1.46	10.7	57.2	17.8	1.47	13.2	71.2	20.0	1.30
203	12.3	56.2	15.1	1.35	12.1	59.0	16.4	1.34	15.3	74.4	18.4	1.28
217	14.6	57.8	13.2	1.31	14.1	62.8	15.5	1.40	16.4	77.4	18.2	1.34
231	15.8	61.0	13.3	1.31	14.7	65.4	15.9	1.44	17.2	81.7	18.8	1.26
249	16.6	62.5	13.1	1.41	15.3	65.9	15.4	1.56	18.7	87.2	19.1	1.34
259	16.6	63.0	13.2	1.38	15.3	68.8	16.4	1.38	19.1	93.7	20.9	1.28
273	16.8	66.4	14.2	1.31	15.6	70.7	16.8	1.54	19.5	94.7	20.8	1.28
287	18.9	69.8	13.6	1.42	16.9	78.3	18.0	1.45	20.4	99.0	21.2	1.28
301	19.5	72.6	14.0	1.30	17.7	79.6	17.6	1.45	20.3	98.5	21.2	1.31
									Suspended swimming 304th day			
315	19.5	72.8	14.0	1.38	17.7	81.4	18.2	1.38	19.8	101.3	22.7	1.22
329	19.4	70.8	13.5	1.36	17.7	77.6	17.0	1.48	19.6	103.6	23.7	1.28
343	20.3	73.0	13.5	1.38	18.6	80.4	17.0	1.40	20.6	104.8	22.9	1.32
557	20.9	74.6	13.6	1.35	18.6	84.1	18.2	1.40	21.6	106.5	22.4	1.28
									Resumed swimming 359th day			
371	21.1	75.3	13.6	1.30	18.8	78.6	16.3	1.43	21.4	105.2	22.2	1.30
385	21.5	78.6	14.2	1.31	19.2	74.1	14.6	1.53	21.7	104.2	21.6	1.27
399	22.1	75.0	12.9	1.36	19.6	80.9	16.3	1.44	21.4	101.9	21.2	1.32
									Discontinued swimming 401st day			
413	22.1	80.1	14.2	1.38	19.8	83.7	17.0	1.46	21.6	105.4	22.0	1.33
427	22.2	82.3	14.8	1.30	20.2	85.9	17.3	1.50	20.6	96.9	20.4	1.37
441	22.2	78.6	13.8	1.42	19.6	89.5	19.0	1.47	21.3	100.3	20.7	1.37
455	22.2	78.0	13.6	1.39	19.0	94.6	21.3	1.44	20.5	100.6	21.7	1.34
469	22.0	81.0	14.3	1.45	19.2	96.8	21.6	1.48	20.4	104.5	23.0	1.36

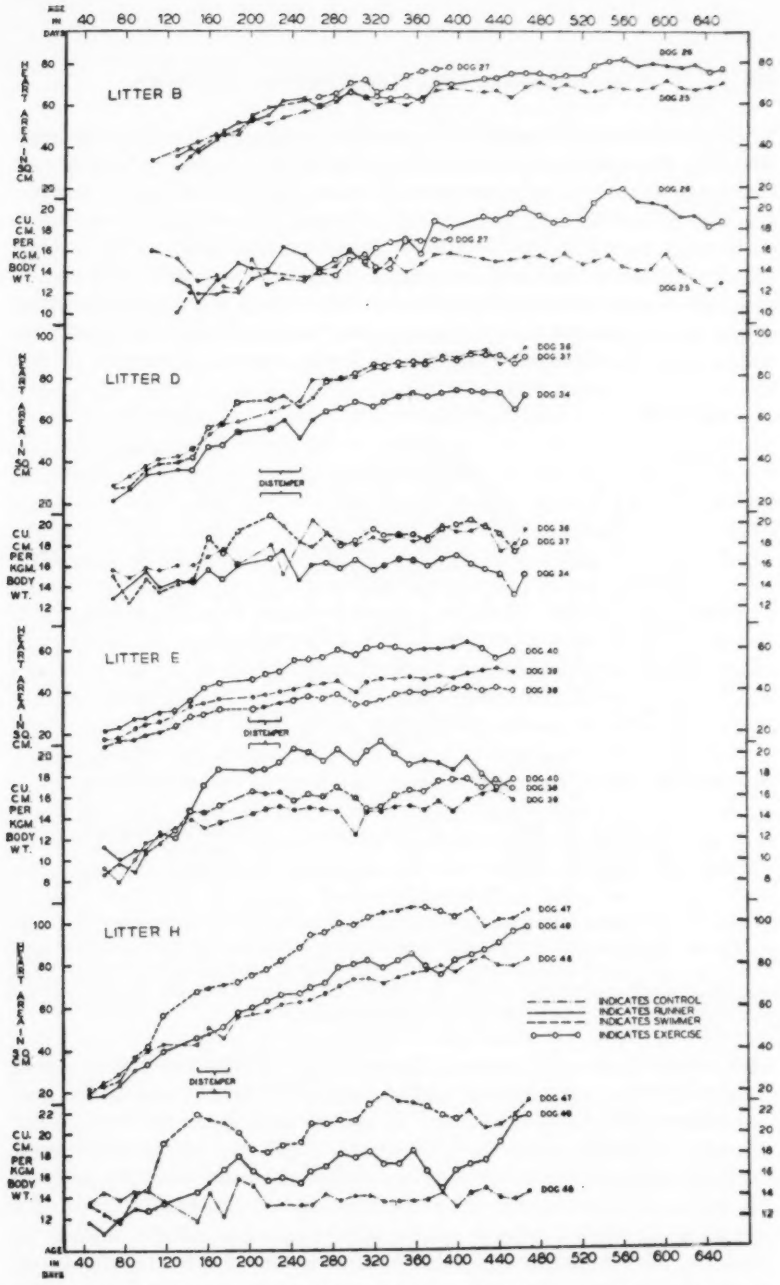

Fig. 1. Graphs of the areas of the heart shadow and of the ratios of heart volume to body weight secured at approximately two week intervals from dogs in litters B, D, E, and H.

largest heart, and the runner the smallest of the three. The tendency shown in the early part of their life period to the 134th day was one of steady growth, in which they retained their relative positions. Exercise was begun the 142nd day. Its effect is shown by a sudden almost immediate rise in the growth curve of both the swimmer and the runner. During the following 75 days dog 34 ran a distance of 520 miles and dog 37 swam 26.5 hours carrying a weight of 2 kgm. Both curves in figure 1 indicate some enlargement. Then exercise was suspended for about six weeks, since all of them, but particularly dog 34, suffered distemper. It was

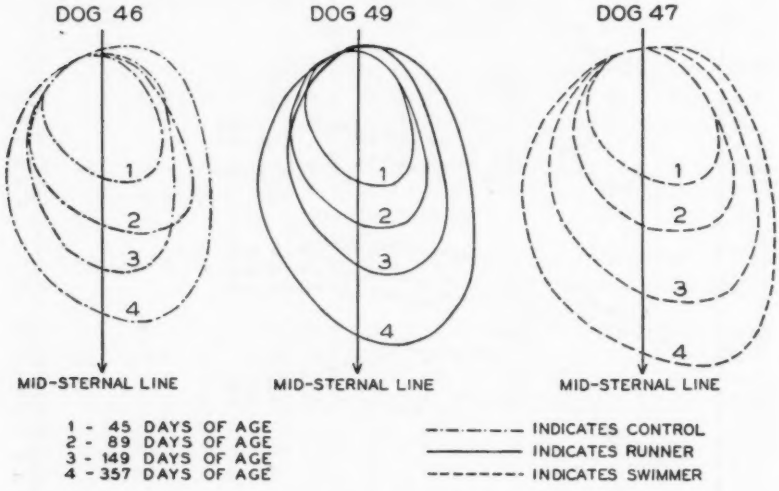

Fig. 2. Successive silhouettes of the heart of individual dogs of litter H traced from Roentgen films at ages indicated and surperimposed on their midsternal axes. Outlines 1 and 2 are from films secured before the beginning of exercise. No particular significance attaches to the shift in the heart axis of dog 46 between the 2nd and 3rd outline. It was not observed in other control dogs.

at this point that one of the controls not included in the data died. During this period the curves indicate a reduction in the cardiac size of the exercised dogs, permitting the control to assume once more the lead. After recovery and resumption of exercise on the 256th day the swimmer again overtook, and for a time exceeded, the control. In the following 207 days the swimmer swam another 102 hours carrying between 4 and 5 kgm. The runner who resumed exercise on the 265th day added 1336.4 miles to his record by the 464th day when the experiment ended. During this period the cardiac size of the control shows about the same rate of enlargement as that of the swimmer. The runner's heart, which was the smallest to begin

with, shows no sign of enlargement, when compared with the control. In this litter the increase in heart size of the swimmer as compared with the control, though marked in the first six weeks, does not persist in the period following the attack of distemper. This peculiar behavior will be considered in the discussion of results.

Litter E comprises dogs 38, 39, and 40 of small short-haired mongrel stock. The data are presented in table 3 and figure 1. In the pre-exercise period their cardiac size shows a steady growth with the runner having the largest heart and the swimmer the smallest. Exercise was begun on the 121st day. Within the first 5 weeks dog 40's heart enlarged considerably as shown in both the area and volume over weight ratio curves in figure 1. He ran a distance of 1759 miles in 239 days. Compared with that of the control his heart size curve indicates progressive enlargement. With the cessation of running on the 360th day, the area curve flattens. He was again exercised from the 415th day to the end of the experiment, covering a distance of 277 miles in the 38 days. During this period the area curve dropped sharply to begin a new rise just before he was sacrificed. This dog was the most enthusiastic runner of the entire series. Dog 38 swam from the 121st to the 206th day of his age a total of 20.5 hours, carrying usually 1 kgm. of weight. There followed a remission of exercise for about a month, due to distemper. On the 239th day exercise was resumed and continued until the 453rd day. During this period he swam 63 hours carrying between 1.8 and 3.2 kgm. of weight. The area curve shows only slight enlargement. This dog was the "runt" of the litter. As noted in the tables his body weight increased but very little in the course of the experiment. The fact that his heart area kept up its relative position with the control though his body weight did not, argues in favor of a relative increase in heart size which is brought out forcibly in the volume over weight curve. If one admits the validity of this reasoning the result of this litter indicates definite enlargement due to exercise, which comes on within five weeks after the beginning of exercise.

Litter H comprises dogs 46, 47, and 49, a mixture of collie, police and airedale stocks. The data appear in table 4 and figure 1. In the pre-exercise period from the 45th to the 90th day of their age, all hearts showed a rapid rate of increase. The control dog's heart was smaller than the swimmer's, but larger than that of the runner. Running was begun on the 90th day and swimming the 96th day. Its effect was marked within three weeks by sharp rises in the areas and volume over weight curves (see fig. 1). This continued to the 143rd day when dog 47 had swam 17.6 hours with a weight of 1.0 kgm., while dog 49 had run 302 miles. Following this the exercised dogs suffered from distemper so that running was discontinued until the 164th day and swimming until the 183rd day. This inactivity reflects itself in a flattening of the area curve of the swimmer, and together with

the fact that his weight increase was not comparably retarded accounts for the sharper drop of the volume over weight curve during this period. From the resumption of running on the 164th day until the 467th day dog 49 ran a total of 1976 miles. His heart which was about 6 sq. cm. smaller than the control's at the beginning of exercise enlarged steadily until at the end it appears larger by about 15.8 sq. cm. From the 183rd to the 303rd day dog 47 swam 41.3 hours with an average of 4.6 kgm. weight. His curves show a progressive rise. Swimming was suspended on the 304th day and resumed between the 359th and 401st days to be again discontinued until he was sacrificed at an age of 473 days. Between the 359th and 401st days he swam a total of 32.5 hours with an average weight of 5.1 kgm. Beginning with the suspension of swimming on the 304th day the curves indicate a retardation of heart growth which turned to an actual regression with marked fluctuations of size during the last swimming and final resting periods. His heart size, which at the beginning of exercise equalled that of the control exceeded it by 28.2 sq. cm. at the end of the major exercise period about the 300th day and then regressed with fluctuations to a point where it exceeded that of the control by only 23.5 sq. cm. at the end. In figure 2 are presented tracings of the cardiac silhouettes of this litter which show that the enlargement in the exercised dogs involved all dimensions symmetrically. These outlines are characteristic of similar ones which we drew for all of the dogs in this series.

Discussion of results. Since the experiment deals with rapidly growing dogs, it is to be expected that the area curve of the heart should also reflect growth by a more or less steady upward trend. The data here presented support this expectation. This growth tendency exhibits itself in its pure form in the pre-exercise period of all our dogs and in the entire experimental period of our control dogs whose exercise was limited to the spontaneous activity of cage life with brief play periods out of doors. In order for a newly introduced factor, in this case extra exercise, to demonstrate its ability to produce modification in such a growing system, its introduction must be consistently followed by divergence from the normal growth curve in either a positive or negative direction. In our experimental procedure this criterion may be satisfied in two ways, as follows: *a*, by a deviation from the trend of the growth curve which was established in the pre-exercise period; *b*, by a deviation from the growth curve of the unexercised control. An analysis of the area curves presented indicates that seven out of eight exercised dogs show an upward deviation within 3 to 5 weeks after the beginning of exercise in fulfillment of the first requirement for establishing our criterion. The exception, dog 49, is the runner in litter H. In this our fastest growing litter exercise was begun in the very week in which, judging from the control, the growth rate began to suffer deceleration. The failure for any deceleration to appear in the area curve

of the runner might be taken to indicate a virtual acceleration. Five out of eight exercised dogs either overtake and surpass their controls, or if already larger, they further draw away from the controls during the period of exercise. Two of the exceptions, dogs 37 and 34 in litter D, show this trend in the twelve weeks between the beginning of exercise and their period of affliction with distemper. Following the illness they do not show enlargement significantly greater than the control. The fact that during the distemper period the heart of the control dog increased in absolute size as well as in proportion to body weight, whereas that of the exercised dogs decreased both absolutely and in proportion to body weight, suggests that the disease which affected these dogs most seriously may account for the exceptions. If this is not a tenable position, then we must regard, especially dog 34, distinctly as an exception, since he was a very good runner having executed 1856 miles in 274 days. The 3rd exception, dog 38, will be considered presently.

The volume over weight ratios presented in our graphs as the cubic centimeter of heart volume, calculated from the area, per kilogram of dog, is virtually the heart quotient of the German literature used extensively in recent years by Herxheimer. As long as the heart grows proportionately to the rest of the body this curve should run parallel to the abscissa. A rising curve indicates the heart is growing more rapidly than the body, whereas a falling curve indicates that the body is growing more rapidly than the heart. With only unimportant exceptions the curves of our control dogs run parallel to the abscissa, whereas those of our exercised dogs show upward trends, the sharper upward trends coming in most cases shortly after the beginning of exercise. It is true that a reduction in body weight with a stationary heart would also show upward trends in this curve. An examination of the weight records and the area curves for the corresponding dates, however, indicates that changes in body weight play a minor rôle in determining the direction of the curves in question. In this connection dog 38 E referred to above as an exception deserves consideration. As already mentioned, he was the "runt" of his litter, and in spite of the best care he weighed consistently and increasingly less than the control, thus at the beginning of the experiment he was 0.7 kgm. lighter, just before exercise began, the difference had increased to 1.2 and at the end of experiment the difference was 3.0 kgm. In spite of this inability to keep pace in body weight with his control, his heart size did not fail to keep up, in fact the curves run practically parallel. In the volume over weight curves this state of affairs leads him to overtake and surpass his control soon after the beginning of exercise thus negating the importance of this exception.

Whenever exercise was discontinued either to favor recovery during the period of distemper or for experimental reasons the curve of the heart area

flattened and the volume over weight curve showed regression of heart volume greater than body weight. This is in accord with the observations of Secher (1923) who found that rats killed immediately after a period of training had larger hearts than those killed several weeks after the cessation of exercise.

If such a rested dog is subsequently again exercised there is at first a marked further reduction in the area of the silhouette followed later by a new rise. On this point our data are yet insufficiently complete. All in all the findings seem to support the hypothesis that the rather uniform progressive growth in exercise is replaced in inactivity by a much fluctuating area as though there were some loss of tone or turgidity in the organ. The rather sharp drop in size immediately on the resumption of exercise could then be interpreted as due to a resumption of tone in an organ whose mass had regressed. The findings of Ackerman (1927) indicating reduction of heart size in a large percentage of veteran oarsmen upon resumption of training fit into this generalization.

The error which is introduced by using a distance shorter than two meters between the target and film is due to the divergence of the rays from the shorter distance acting to magnify the heart shadow. The degree of this magnification at any given target distance is further proportional to the distance between the object photographed and the film. Thus from theoretical calculations it is found that the shadow of an object at a distance of 10 cm. from the film when photographed at a target distance of 6 feet is 11.6 per cent smaller than when the target is at a distance of 89 cm., while with a distance of 15 cm. between object and film this figure is 18 per cent. Though one cannot determine with accuracy the level of the heart within the chest our estimate from many measurements would place this distance at about 15 cm. in our larger dogs. We checked these estimates by photographing a number of dogs at the short and long target distances and found that the six foot distance gave differences in heart areas which were quite constant for a single dog and which varied from 14 to 22 per cent in different animals. There is no doubt that as the dogs grew the chest depth and therefore the distance between the heart and film increased. In view of these uncertain and changing factors we have deemed it unwise to apply correction factors to our measurements of the shadows. Since our litter-mate dogs were usually of comparable form and proportions this increasing distance no doubt leads to progressively greater enlargements of the heart of the control as well as of the exercised animal, and therefore, though the absolute values may be somewhat distorted, the growth trends and exercise effects pointed out, may be considered valid. Further, the marked change in trend of growth of the cardiac area which follows promptly on the beginning of exercise cannot be ascribed to anything but changes in the heart itself. In this connection it is in place to refer once more to dog 38

E, the runt whose heart area seemed irresponsive to exercise. As already mentioned he failed to keep up in bodily dimensions, including depth of chest, with his litter mates. In these peculiarities may lie further explanation for the failure of this heart to display the characteristic exercise response which postmortem findings revealed was present.

Thörner has recently (1930) reported a relative lengthening of the heart in trained dogs in comparison with unexercised controls who during the same period developed rounder heart shadows. He attributes this to an increase in the long diameter at the expense of the shorter, and expresses it in a length to breadth ratio. Thus he claims that a period of training increases this ratio. We calculated this ratio for all of our dogs. The data appear in the tables. For one litter, namely, E, we have also graphed these ratios (see fig. 3). We have also traced cardiac silhouettes of representative stages in the development of our dogs' hearts directly from the film (see fig. 2). In none of these methods of analysis do we find any

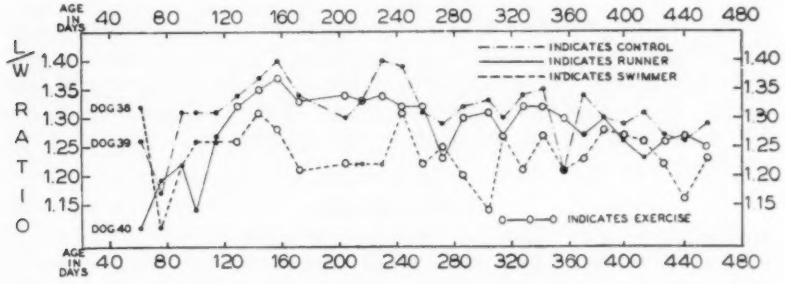

Fig. 3. The length to width ratios plotted for dogs in litter E.

confirmation of Thörner's view. Though it is true that the hearts do not grow uniformly and for weeks at a time may show an excessive increase in one or the other axis we have been unable to associate such specific form development with an exercise program.

SUMMARY

1. Biweekly roentgenographic records of the cardiac silhouette of twelve male dogs from four litters were measured for area, greatest length, breadth, and transverse dimension. The length to breadth and volume to weight ratios were calculated. These determinations are presented in the form of tables, graphs, and outline figures.

2. Two criteria for the establishment of the effect of exercise on a growing heart curve are developed and the data analyzed accordingly.

3. The conclusion seems warranted that strenuous exercise leads to an enlargement of the heart of growing dogs which appears within 3 to 5 weeks from the beginning of exercise.

4. From the heart volume to body we[illegible] it appears that this cardiac enlargement is not paralleled by [illegible]o ıding increases in body weight.

5. Variations in the degree of enlargeme[illegible] the heart found in different animals seem related to the degree of [illegible]tion and the willingness to exercise exhibited by the animal.

6. The length to breadth ratio shows [illegible]ed but irregular changes in the control as well as the exercised dogs. [illegible]ıe data, therefore, refute the position that exercise causes a relatively [illegible], eatc increase in the long diameter.

7. There are indications pointing to regression of the heart size when exercise is discontinued. There is also son e evidence that a second period of training following an inactive season is marked, initially, by a further decrease in heart size. A possible explanation fc this phenomenon is discussed.

We take this opportunity to express our thanks to the Department of Physiology of the University of Chicago and to Prof. A. J. Carlson for permission to use the roentgenographic equipment of the University in this study. To Dr. Paul C. Hodges of the Albert Mer itt Billings Hospital are due our thanks for his valued help and counsel on matters of x-ray technique.

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STUDIES IN THE PHYSIOLOGY OF EXERCISE

IX. The Chronic Effects of Running and Swimming Exercise on the Hearts of Growing Dogs as Determined Electrocardiographically[1]

ARTHUR H STEINHAUS, ROBERT W. BOYLE AND THOMAS A. JENKINS

From The Young Men's Christian Association College Laboratory for Physiologic Research in Physical Education, Chicago

Received for publication September 9, 1931

The electrocardiogram yields undisputable data on cycle length and various other time intervals within the cycle. The gram has also been used to calculate the electrical axis with the hope of detecting preponderance of one or the other ventricle. From general inspection and descriptive comparisons of the relative heights and configurations of the various complexes the gram has served also to designate certain classes of conditions. Of these only the measures of duration of various cycle phases have remained unchallenged as significant indices of what they propose to measure.

In 1925 Meyer observed a very much prolonged P-Q interval in one athlete. Since then Hoogerwerf (1929), Csinady (1930) and Thörner (1930) have made similar observations on a large number of athletes and trained dogs. Hoogerwerf and Thörner also reported greatly increased cycle lengths, the former having observed pulse rates as low as 30 per minute in Olympic athletes.

As further signs of the trained state these two workers have described a reduced P wave and an increased T wave. Schenk (1925) and Kostjukow and Reiselmann (1930) have reported similar changes in the T wave of laborers. U waves have been found by Hoogerwerf in only two men both of whom were Olympic champions, while Csinady also discovered two which his observations lead him to conclude are associated not with particular fitness but rather with long intensive athletic careers. Hoogerwerf's electrocardiograms indicate that most Olympic athletes suffer either left or right ventricular preponderance, the normal condition being rare. Only the work of Thörner was controlled in an experimental sense.

[1] A grant from the Carnegie Corporation of New York has aided the conduct of this study. A special grant from the American Association for the Advancement of Science covered the cost of films and processing chemicals.

This worker exercised two male dogs whilst holding for each a littermate brother as unexercised control. One pair was ten months, the other two years old at the beginning of exercise. He secured electrocardiograms by lead I from each dog several times before the beginning and again at the close of the exercise period.

EXPERIMENTAL METHOD. The animals used for these observations were the ones employed in the roentgenographic studies reported in the preceding paper to which the reader is referred for all details of management, etc. The puppies were trained to lie quietly on their right sides before any attempts were made to secure electrocardiograms. These were taken after thirty to forty-five minutes of complete rest in the post-absorptive state on a Victor Portable Electrocardiograph[2] in all three leads at regular biweekly intervals throughout the pre-exercise and exercise periods. The dog's legs were shaved and connected with pliable lead amalgam strip electrodes wrapped around the leg previously soaked with a saturated solution of table salt. A large number of measurements including deflection magnitudes of all the major waves as well as time intervals, were made. After a great deal of testing was done in the hope of finding some combination of measures which would reflect growth trends and exercise effects, all were discarded with the exception of the height of the R wave in the three leads for calculating the electrical axis and the P-R and R-R intervals for detecting changes in conduction rates and cycle lengths. We calculated the electrical axes according to the directions given by Lewis (1925, p. 107) in which Einthoven's formulae, $\tan a = \frac{2e^2 - e^1}{e^1\sqrt{3}}$ and $\tan a = \frac{e^2 + e^3}{(e^2 - e^3)\sqrt{3}}$ are employed. We secured values for e in the three leads by averaging in the gram from each lead, the heights of all of the R waves secured in that lead. In most cases these average values fulfilled the requirements of the formula $e^3 = e^2 - e^1$. Each angle, a, was then calculated from the data of leads I and II. and from leads II and III. The values so secured which usually checked within 5°, were averaged and this average was graphed as the electrical axis of the heart for that day. The P-R and R-R intervals were measured by the aid of a hand lens to 0.1 mm. Repeated checks indicated an error in reading which did not exceed ± 0.1 mm. This is equivalent to ±0.004 second. We have used the designation P-R, as defined by Pardee (1928, p. 21) to mean the time from the beginning of the P wave until the QRS group begins.

RESULTS. Our data secured by the methods outlined above are presented in figure 1 which portrays the electrical axes of the twelve dogs at biweekly intervals plotted on a time scale indicating the dog's age in days;

[2] The machine was put at our disposal through the courtesy of the General Electric X-Ray Laboratories of Chicago.

in figure 2 in which P-R intervals are plotted against R-R intervals in each case for the last two or three periods immediately preceding exercise (indicated by dots) and for two or three periods after the dog had been exercised to a highly trained state (indicated by crosses); and in table 1 which compares mean values for the P-R and R-R intervals in exercised and unexercised dogs.

DISCUSSION OF RESULTS. *Electrical axes.* The fact that our dogs were rested and electrocardiographed while lying on their right sides no doubt introduced some opportunity for shifting of the heart toward the right side. We have taken grams on a number of dogs successively in the right lateral and supine positions and calculated the axis angle for both positions. The differences resulting from the two calculations though quite constant for a given dog on successive days, varies a great deal from dog to dog. In some cases the angles calculated for the two positions checked exactly while in other cases they differed as much as 15°. We feel certain that the lateral position has real virtue not only because it is much easier to train a dog to assume a constant and quiet position while lying on his side, but also because there is considerable danger of the heart sagging to the left or right side when it lies near the top of the chest with the dog in supine position, especially if this position is held with difficulty. The extent and importance of such changes have recently been pointed out by Nathanson (1931). A study of figure 1 reveals with but a single exception no general trend in axis shift associated with exercise. The exception is dog 38 who shows, soon after the beginning of exercise, a definite drawing away from his control in the direction of a reduced angle which should indicate left hypertrophy. The autopsy findings on this dog (see the succeeding paper by Steinhaus, Hoyt and Rice) revealed marked right hypertrophy. The explanation for this difference must be sought in a secondary rotation of the heart as described by Meek and Wilson (1925) and Nathanson (1931). Failure for any of the hearts of other exercised dogs to show preponderances is not out of harmony with the very small left sided hypertrophies discovered at autopsy and the conclusions of Herrmann and Wilson (1921) and Meek and Wilson (1925), namely, that only marked unilateral hypertrophies record their presence in altered electrocardiographic complexes. The very marked temporary shiftings of the electrical axes in several dogs, notably the control 25B, puzzle us. We have been unable to detect comparable shifts in the anatomic axes of the heart in our x-ray records taken during these periods and there is no indication in the general health or food-consumption record to suggest any ailment. There is left the possibility of unequal growth or development in the two sides of the heart.

Cycle length and P-R interval. Dawson (1919), Scott (1924), Damez et al (1926), Yandell Henderson et al, (1927) and Bramwell and Ellis (1929)

are among those who have reported slower pulse rates in athletes, or in consequence of a season of athletic training. Hoogerwerf (1929) reports pulses as low as 30 per minute in Olympic athletes and Katz (1928) described such a case of bradycardia which recovered after two months' rest. On the other hand Ackermann (1927) found reduced pulse rates in only 65 per cent and 58 per cent of individuals in two groups trained in rowing and Gemmill et al. (1930) report no significant changes in basal pulse rates in consequence of several weeks of training on a bicycle ergometer. Nor

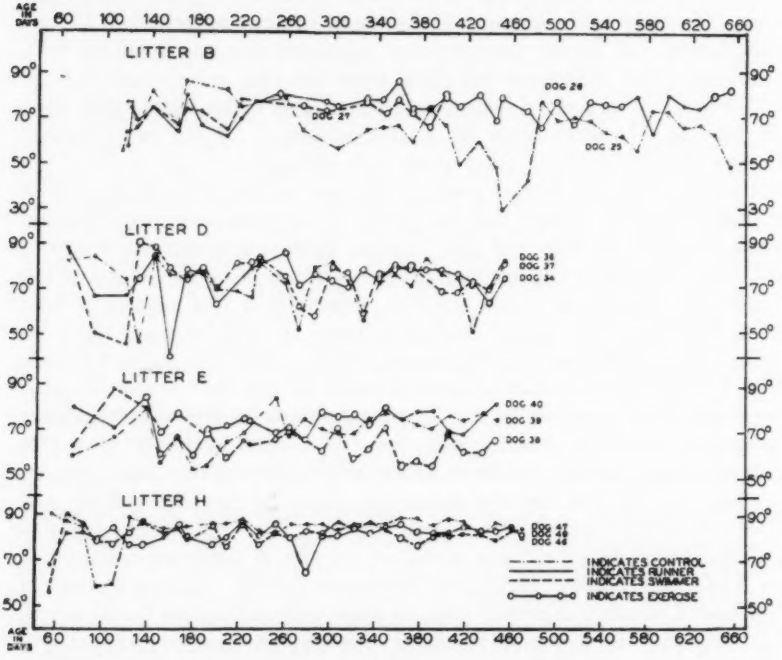

Fig. 1. The angle of the electrical axis, calculated from bi-weekly electrocardiographic records, is plotted on a time scale indicating the dogs' age in days.

were we able in this laboratory to discover significant changes in the basal pulse rate of highly trained dogs which we used to study the effects of exercise on basal metabolism over long periods. Our findings on pulse rate (R-R) from analyses of 1202 individual cycles in twelve dogs are represented graphically in figure 2. For each dog we have here spotted the cycle values of grams secured during a number of weeks before exercise in such a way that they can be compared with similar data secured in an advanced stage of training. Here also can be read the P-R intervals of the

corresponding cycle lengths. It is a bit disappointing, at first thought, that there should be no marked and consistent difference in the picture representing exercised and control dogs. A cursory inspection of this chart indicates that there are marked changes in both P-R and R-R in the control dogs as well as the exercised ones. In order to determine the kind and extent of these changes which may be attributed to exercise it is neces-

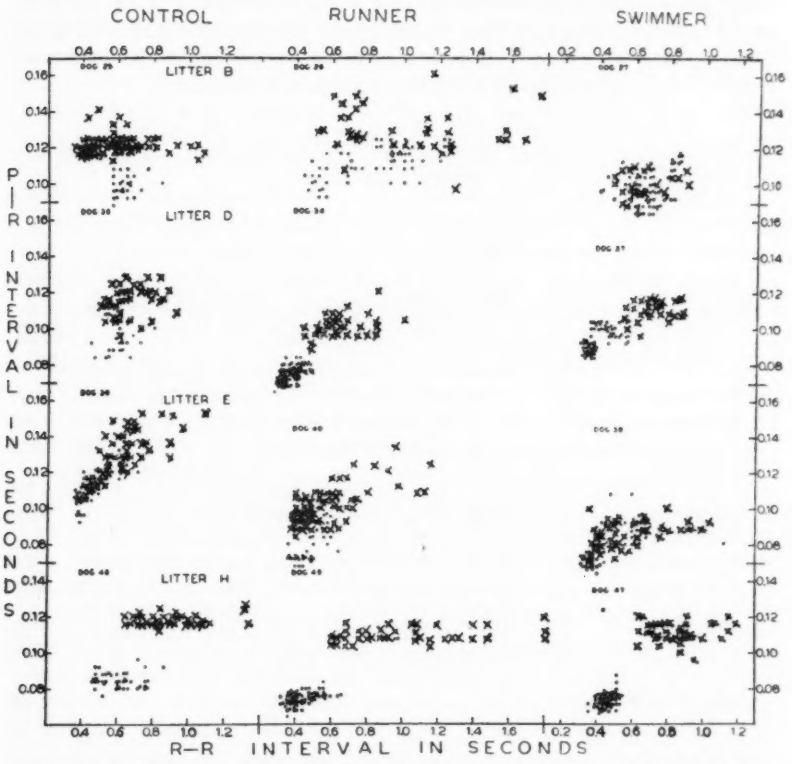

Fig. 2. Scattergrams of P-R against R-R for each dog before and after training. The dots indicate observations in the pre-exercise period while crosses represent the trained state.

sary to devise some way of subtracting from the changes observed in the exercised animals the amount of similar change which merely "growing up" induced in the controls. Although we realize keenly the limitations of a small body of data subjected to this type of treatment we have made certain calculations which we believe help to isolate the exercise effect. These are presented in table 1. From the mean values for P-R and R-R

of the periods before and during training we have determined the changes which these values underwent during the periods in question. We have subtracted the amount of change found in the control from the amount of change found in the exercised dog and assume that this difference is a measure of the change which may fairly be attributed to exercise. We have averaged these differences for runners and swimmers. The figures indicate that running and swimming increase the cycle length (R-R) by 0.147 and 0.119 second respectively. In the case of the conduction time (P-R) an increase of 0.001 second may be attributed to running while swimming results in a net reduction of 0.007 second in this element.

TABLE 1

Mean P-R and R-R intervals before and after training with calculations to eliminate changes due to growth

DOG	P-R				R-R			
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
	Before training	After training	Change (2)-(1)	Portion of change due to exercise	Before training	After training	Change (6)-(5)	Portion of change due to exercise
	seconds	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*	*seconds*
25 B Control	0.104	0.118	0.014		0.611	0.560	−0.051	
26 B Runner	0.111	0.126	0.015	0.001	0.817	1.008	0.191	0.242
27 B Swimmer	0.098	0.100	0.002	−0.012	0.658	0.677	0.019	0.068
36 D Control	0.092	0.115	0.023		0.598	0.654	0.056	
34 D Runner	0.075	0.101	0.026	0.003	0.374	0.640	0.266	0.210
37 D Swimmer	0.095	0.110	0.015	−0.008	0.405	0.709	0.304	0.248
39 E Control	0.113	0.134	0.021		0.458	0.656	0.198	
40 E Runner	0.083	0.101	0.018	−0.003	0.484	0.540	0.056	−0.142
38 E Swimmer	0.078	0.085	0.007	−0.014	0.448	0.578	0.130	−0.068
46 H Control	0.085	0.118	0.033		0.608	0.894	0.286	
49 H Runner	0.074	0.110	0.036	0.003	0.444	1.007	0.563	0.277
47 H Swimmer	0.074	0.111	0.037	0.004	0.461	0.877	0.416	0.230
Average all Runners				0.001				0.147
Average all Swimmers				−0.007				0.119

Most of the workers in this field whom we have cited, agree with Schenk (1925) in attributing changes in cycle length and P-R interval to a vagotonia brought on by the training period. The observations originally made by Herxheimer and recently confirmed by Kauf (1926) to the effect that 1 mgm. of atropine does not accelerate the pulse of athletes as it does that of non-athletes, favor this view. According to present opinion (Starling, 1930, p. 747) the action of the vagus is confined to the auricle and is a threefold one, namely, 1st, reduced excitability of the S. A. node which leads to a slowing to the rate; 2nd, reduced contractility of auricular musculature resulting in a weaker auricular contraction; and 3rd, reduced

conduction in the A-V bundle which finds expression in an increased P-R interval.

Results on experimental animals permit one to assume that not all of these actions need necessarily be involved at once and to the same extent. Thus it is possible to visualize a change of rhythm with or without marked changes in conduction or in fact any other combination of these possibilities. Further, the slowing of the pulse rate would permit for more complete recovery of the auricular tissue so that the conduction rate within the auricular wall would be increased thus shortening the first phase of the P-R interval (auricular conduction phase). While the second phase which records the time for passage over the A. V. bundle may or may not be lengthened depending on whether the vagus effect involves this tissue. The P-R as measured on the gram actually reflects the algebraic sum of the effect of these two factors.

Projected against this background of reasoning it would appear that both our runners' and swimmers' hearts with the exception of those in litter E, were under the influence of vagus tone. This exhibits itself by an increase in cycle length in both groups. The greater increase is found in runners. The P-R interval is also lengthened though only slightly in the runners; but in the swimmer it is actually shortened. It seems probable that the lengthening of the cycle is enough to enhance recovery in the auricles sufficient to permit improvement in the rate of conduction through the auricular wall. A vagus tone which affected the S. A. node particularly, would provide the necessary conditions to make this interpretation valid. Dogs 38 and 40 in litter E deserve special attention. Whereas all other exercised dogs showed increases in cycle length in excess of their controls, these do not; so that at the height of training their pulse rate has slowed less than that of their control, dog 39. These dogs also show smaller increases in conduction time than does the control. Dog 27 B though showing an increase in R-R in the data here presented, gave a very interesting subsequent history. In the following weeks his R-R interval gradually shortened until it was actually shorter than before the beginning of exercise. He was an excellent swimmer. However, on the 399th day, his exercise was cut short because of obvious physical embarrassment. On the 402nd day he collapsed and died shortly after the exercise was begun. Autopsy revealed no abnormal signs beyond an apparently small aortic diameter and thickened aortic walls. The electrocardiograms which up to this point had indicated shortening P–R and R-R intervals, suddenly showed much longer time values in the grams taken on the day before his death. The possibility of the existence of a relation between these alterations in interval lengths and his failing heart has suggested itself to us as a strong likelihood and one that should be kept in mind in the event that succeeding studies may provide opportunity for confirmation. In the

face of these facts, we have reported in table 1 the data from his grams before the R-R interval began to shorten abnormally. The fact that this dog suffered an attack of distemper which temporarily paralyzed and blinded him during his pre-exercise period, has given us further reason to think that in this case we may have been dealing with a defective heart. The contours of all his electrocardiograms appear to be normal.

We have examined our grams also for the purpose of detecting changes in the amplitude of the P and T waves as reported by other observers. In this we have been unsuccessful. The same exercised dog may show small P and large T at one time and a week later show large P and small T while controls may show the deflections which have been attributed to trained dogs and men. On the other hand we have also many records which conform to the contours described by Hoogerwerf for man and Thörner for the dog. The findings are, however, far from consistent.

The greater spread in R-R interval times, indicating respiratory arrhythmia, in the trained dogs, which is apparent in our figure 2, is in agreement with the findings of Thörner and is in itself further evidence for the presence of a greater vagus tone in the trained state.

SUMMARY

1. From electrocardiograms secured at biweekly intervals from twelve growing dogs, in periods of inactivity and training, electrical axes have been calculated and P-R and R-R intervals measured. The findings on control and exercised dogs are compared in two graphs and a table. The grams were secured under basal conditions from dogs trained to lie on their right sides.

2. The calculations of electrical axes do not reflect the minor left-sided preponderances found on autopsy. In the case of one with marked right-sided hypertrophy the electrical axis showed a persistent counter clockwise rotation.

3. From P-R and R-R measurements treated to separate out changes due to "growing up" there is evidence that training is characterized in a majority of dogs by an increase in vagus tone. This does not always exhibit itself in increased conduction time (P-R). The reasons are discussed. Two of our dogs who failed to show increases in R-R greater than their control support the position reported also by others, that the trained state is not always accompanied by a slowing pulse.

4. The data do not support the view that the trained state is consistently marked by a reduced P wave and a pronounced T wave.

5. Trained dogs show an increased tendency toward respiratory arrhythmia.

We are indebted to Dr. L. N. Katz for his valued counsel in the interpretation of our data.

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STUDIES IN THE PHYSIOLOGY OF EXERCISE

X. The Effects of Running and Swimming on the Organ Weights of Growing Dogs[1]

ARTHUR H STEINHAUS, LORIS A. HOYT AND HUGH A. RICE

From The Young Men's Christian Association College Laboratory for Physiologic Research in Physical Education, Chicago

Received for publication September 9, 1931

The actual weights of organs secured at autopsy are without doubt among the most dependable data from which to draw conclusions concerning the effects of exercise. In this paper are presented such findings from eleven of the twelve dogs which provided the roentgenographic and electrocardiographic data of the two preceding papers. The heart weights and ratios here reported confirm the findings there reported. In addition this paper presents data on leg musculature, leg skeleton, eyes, lungs, liver, kidney, spleen, thyroid, adrenals and testes of exercised dogs compared with their unexercised littermates.

Experimental methods. For the details of general management, feeding and exercising the reader is referred to the paper describing the roentgenographic findings on these animals (Steinhaus, Kirmiz and Lauritsen, 1932). The members of a litter were in each case sacrificed in rapid succession on a single day after food had been withheld for thirty-six, and water for twenty-four hours. Under light ether anesthesia a cannula was inserted into the left carotid artery and bleeding encouraged by massage and passive movements of the head, trunk and limbs while the dog was held vertically with head downward. The chest was quickly opened and the heart removed. This was followed by the other internal organs in a regular and fixed order. Thus all of the fresh organ weights were secured within twenty minutes after the chest was opened and all were subject to approximately similar weight loss by evaporation. The heart was weighed after freeing of pericardium and blood clots with veins cut at auricles and arteries cut just above the valves. The auricles were then removed, ventricles weighed, valves examined and tested by water under pressure, ventricle volume determined by the liquid displacement method using physiological salt solution, and finally hardened

[1] A grant from the Carnegie Corporation of New York has aided the conduct of this study.

in 10 per cent formalin. After seven to ten days the ventricles were separated by the method of Herrmann (1925) and the weights of left and right ventricles corrected to fresh weight. The lungs were weighed with bronchi cut flush with the lung surface. All organs were carefully trimmed of external fat and communicating vessels cut at the organ surface. The vas deferens was transected at the level of the top of the testis. After skinning and removing the superficial fat the right forelimb and left hind limb were removed from the carcass with all of the muscles engaged in locomotion dissected at their origins on the trunk. In the forelimb this included the scapula and all its muscles. After weighing the extremity, all of its muscles above the ankle were carefully removed. The weight of both limbs, after this treatment, was multiplied by two and recorded as "leg skeleton." The "leg musculature" is the difference in the weights before and after removing the musculature in the two extremities, also multiplied by two. The reason for not determining muscle weights directly for all of the extremities was due to our desire to keep the joints of two extremities unmolested for the purpose of testing ligament strengths. Gross body weights are accurate to within fifty grams while all other weights were determined to 0.1 gram. All organs were examined and note made of any abnormal findings. The leg bones were sectioned at several levels and inspected for bone marrow changes.

RESULTS. In table 1 are presented certain organ weights and organ weight to body weight ratios for four unexercised dogs, for four who were exercised by running and for three who were exercised by swimming. The following special abbreviations are used in the table: B. W. for body weight, H. W. for heart weight, L. M. for leg musculature, L. V. and R. V. for left and right ventricle respectively.

Averages have been calculated for each activity group, i.e., controls, runners, and swimmers. The percentage difference between the average figures of runners and of swimmers compared with controls, has been calculated to show the percentage deviation of the exercised groups from the control which is assumed to be 100 per cent.

A correction factor which we have applied to certain of these percentage figures needs special explanation. There is no question but that subcutaneous and other extra-organ fat deposits account in large part for the heavier body weight of our control dogs. Since the organs which we have weighed with the possible exception of leg musculature do not share these fat deposits it is clear that organ to body weight ratios will always tend to give higher quotients in the thinner animals whether or not there has been actual hypertrophy. Since this distortion is proportional to the difference in body weight we have concluded that percentage differences in the cases of organ to body weight ratios will come considerably nearer expressing actual organ differences if the percentage figure is corrected by an amount equal to

TABLE I. AUTOPSY FINDINGS COMPARING ORGAN WEIGHTS OF EXERCISED AND UNEXERCISED DOGS

LITTER	DOG	KIND AND AMOUNT OF EXERCISE	AGE days	BODY WEIGHT kgm.	LEG MUSCLES gms.	HEART WEIGHT gms.	SPEC. GR. of VENTRICLE	A LM/BW	A LEG SKIN/BW	A EYES/BW	A HW/BW	A HW/LM	T LV/RV	I LUNGS/BW	I LIVER/BW	O KIDNEYS/BW	S SPLEEN/BW	THYROID/BW	ADRENALS/BW	TESTES/BW
B	25	NO EXERCISE	660	16.50	4568	119.72	1.068	.277	.0467	.000642	.00726	.0262	1.449	.00572	.0198	.00223	.00160		.0000848	.00172
	26	RAN 1830 MILES		14.90	4210	141.50	1.037	.283	.0515	.000710	.00950	.0336	1.493	.00722	.0233	.00361	.00201		.0000593	.00220
D	36	NO EXERCISE		19.25	5200	157.30	1.043	.270	.0547	.000649	.00817	.0303	1.421	.00711	.0208	.00367	.00211	.0000571	.0001090	.00202
	34	RAN 1836 MILES	467	16.60	4515	114.40	1.014	.272	.0560	.000717	.00813	.0234	1.431	.00668	.0250	.00374	.00211	.0000542	.0000873	.00192
	37	SWAM 128 HRS.		18.90	5169	169.30	1.034	.274	.0568	.000624	.00896	.0328	1.533	.00410	.0251	.00461	.00120	.0000635	.0000847	.00138
E	39	NO EXERCISE		9.10	2372	69.50	.997	.261	.0531	.001150	.00763	.0293	1.426	.00717	.0216	.00384	.00213	.0001100	.0000879	.00152
	40	RAN 2036 MILES	457	10.85	3036	98.60	1.051	.280	.0494	.001010	.00909	.0325	1.492	.00589	.0236	.00379	.00168	.0001290	.0000645	.00128
	38	SWAM 83 HRS.		6.50	1759	67.20	1.062	.271	.0533	.001450	.01034	.0375	1.277	.00805	.0282	.00491	.00209	.0001840	.0001850	.00168
H	46	NO EXERCISE		21.45	5212	143.20	1.050	.243	.0526	.000604	.00668	.0275	1.493	.00628	.0190	.00345	.00186	.0000280	.0000723	.00159
	49	RAN 2278 MILES	473	19.05	5330	166.00	1.094	.280	.0662	.000625	.00840	.0300	1.574	.00842	.0136	.00364	.00291	.0000682	.0000919	.00109
	47	SWAM 91 HRS.		19.75	5367	179.20	1.052	.272	.0560	.000387	.00907	.0334	1.526	.00467	.0229	.00421	.00190	.0000685	.0000962	.00085
CONTROLS AVERAGE				16.575	4388	122.43	1.039	.263	.0502	.000761	.00744	.0283	1.447	.00655	.0262	.00335	.00193	.0000650	.0000885	.00166
RUNNERS		AVERAGE		15.350	4273	128.67	1.049	.279	.0558	.000766	.00878	.0304	1.498	.00703	.0214	.00370	.00218	.0000838	.0000788	.00162
		% dif. Controls as 100%		-7%	-2.6%	+5.0%	+0.9%	+1.7%**	+5.0%*	-6.4%*	+11.0%*	+5.4%**	+3.0%	+0.6%*	-1.0%*	+3.0%*	+6.0%*	+20.0%*	-21.0%*	-2.0%*
SWIMMERS		AVERAGE		15.050	4098	138.57	1.049	.272	.0554	.000887	.00946	.0342	1.530†	.00541	.0254	.00458	.00175	.0000936	.0001220	.00130
		% dif. Controls as 100%		-10%	-7.0%	13.0%	+0.9%	+0.4%**	0.0%*	+6.6%*	+12.0%*	+14.0%**	+6.0%	-27.0%*	+16.0%*	+26.7%*	-20.0%*	+34.0%*	+28.0%*	-31.7%*

*PERCENTAGES CORRECTED FOR DIFFERENCES IN BODY WEIGHT OF CONTROLS AND EXERCISED DOGS. FOR RUNNERS THIS CORRECTION IS -7.0%. FOR SWIMMERS IT IS -10.0%.

**PERCENTAGES CORRECTED FOR DIFFERENCES IN LEG MUSCULATURE WEIGHT OF CONTROLS AND EXERCISED DOGS. FOR RUNNERS THIS CORRECTION IS -2.6%. FOR SWIMMERS IT IS -7.0%.

†EXCLUSIVE OF DOG 38E WHO SHOWED MARKED RIGHT VENTRICULAR HYPERTROPHY.

the percentage difference in the body weights. We have therefore corrected by −7.0 per cent and −10.0 per cent all percentage difference figures for runners and swimmers, respectively, in which body weight appears as denominator of the ratio. Likewise for similar reasons we have made corrections of −2.6 per cent and −7.0 per cent for runners and swimmers respectively in the percentage difference figures for those ratios which have muscle weight in the denominator. A logical extension of this reasoning led us to change the sign of the leg muscle weight correction in the one ratio where this item appears in the numerator, i.e., $\frac{LM}{BW}$. In this instance there was of course also the regular correction for body weight differences. The percentages which have been corrected in this wise are appropriately designated by references to footnotes in the table.

Discussion of results. *Body weight.* With but one exception (40E) the exercised dogs are lighter than their controls. Differences in the amount of fat no doubt account largely for these variations though in the case of 38E, a swimmer, who weighed almost 30 per cent less than his control, constitutional factors must have entered in.

Leg musculature. The average weight of leg musculature in the exercised animals is actually less than that of the controls. In litters E and H, however, the exercised dogs have heavier muscles. All of the $\frac{LW}{BW}$ ratios show moderate enlargement of muscles in the exercised groups but when corrected in accord with the rule adopted above there is no indication of true hypertrophy. Both Külbs (1912) and Thörner (1930) report comparable findings. Although his ratio favors the musculature of exercised dogs, Thörner's figures for absolute weight run as do ours. He has not corrected for differences in body weight as have we.

Eye balls. The $\frac{eye}{BW}$ ratios show only comparatively insignificant differences of −6.4 per cent and −2.0 per cent in the runners and swimmers respectively. It is interesting to note that Hatai (1915) found that the eyeballs of exercised rats weighed 9.45 per cent less than those of normal rats.

Leg skeleton. In runners the leg skeletons averaged 5 per cent heavier than controls while in swimmers there was no difference. These figures as well as those for the eye are probably without meaning beyond indicating the extent of the range of insignificant variations.

Heart weights. The absolute weights of exercised dogs' hearts exceed those of the controls by 5 per cent and 13 per cent for runners and swimmers, respectively. This is brought out more forcibly in the $\frac{HW}{BW}$ ratio in which even after correction for the lighter body weights of the exercised dogs the

runners show 11 per cent and the swimmers 17 per cent greater quotients. The $\frac{HW}{LM}$ ratio after correction for the lighter leg musculture of exercised animals still shows 5.4 per cent and 14.0 per cent in favor of runners and swimmers respectively. These figures are in accord with those of Külbs (1906), Grober (1908) and Thörner (1930) gained from dogs and those of Hatai (1915), Secher (1921) and Borovansky (1930) on rats and Hiramatsu (1919) on rabbits. That the heart hypertrophies at a more rapid rate than do the skeletal muscles, if the latter hypertrophy at all, is the acknowledged criterion of true hypertrophy as defined by Bruns (1909): "Gibt es bei gesunden Menschen eine Arbeitshypertrophie des Herzens, d.h. eine unverhältnissmassig starke Zunahme der Herzmasse durch Körperarbeit in Vergleich zür ebenfalls zunehmenden skelettmuskulature, oder besteht der Satz zu recht, dass bei gesunden körperlich arbeitenden Menschen die Masse des Herzmuskels der Körpermuskulature entspricht?" From observations on eight dogs this last named worker concludes that heart and muscle hypertrophy simultaneously and proportionately in exercise. The method of exercising employed by him was rather unique. The dogs were required to walk for 30 to 45 minutes on an electrically driven treadmill pitched at a 50 per cent grade, and operated at the rate of one kilometer in 15 minutes. There is a great deal of difference between this form of exercise and the free running in wheels or on low-pitched treadmills, employed by other workers.

In 1924 Herxheimer advanced the hypothesis supported by a large datum gained from expert athletes, that exercises of strength, speed or big effort with frequent rests induce hypertrophy of skeletal muscle and heart to the same extent, whereas endurance work does not develop skeletal musculature but causes cardiac hypertrophy since in it the heart is taxed while the skeletal muscles are actually working at a slow rate. This hypothesis is supported by the observations of Kohlrausch (1929) who found more protein deposition in animals trained to uphill and pulling exercise than in those trained in distance running. Since most forms of exercise employed with laboratory animals are of the endurance type we should expect the results from this form of work to compare with the findings on long distance runners among human athletes. It is possible that in this light the autopsy findings by Hirsch (1899) on normal men and by Dibbelt (1917) on soldiers killed in action, namely, of a constant relation between heart and skeletal musculature, as well as the work of Bruns on dogs and a host of others who have studied the hearts of human athletes by x-ray, may all be resolved into a unified whole.

Though the specific gravity of the ventricles averages higher for exercised dogs than for controls, this does not represent a consistent finding for all litters. Thörner (1930) found higher specific gravities for the hearts of

both of his exercised dogs. In all of the exercised dogs with one exception the $\frac{LV}{RV}$ ratio shows slight left ventricle preponderance which, compared with the control, is +3 per cent and +6 per cent for runners and swimmers respectively. This is in agreement with the reports of Külbs, Grover, Borovansky and others. The exception, dog 38-E, showed a very marked right preponderance. A gross inspection of this swimmer's lungs revealed many little bubbles on its surface which disappeared when the lungs were inflated. If this may be taken as a sign of lung emphysema, this case would fall in line with the observations of Hiramatsu (1919) who found right hypertrophy associated with an acute exercise emphysema in rabbits. The fact that this dog was very small and always swam with signs of respiratory embarrassment, is not an irrelevant observation.

Before leaving the discussion of heart data attention should be called to litter D in which the control dog 36 shows an unusually large heart. Reference has already been made to the troublesome nature of this case in the paper on the roentgenogram findings where it was hard to decide whether the runner's heart failed to enlarge or whether the control's heart hypertrophied in response to some undiscovered stimulus. Inspection at autopsy revealed an unusually large and flaccid pulmonary artery which tore very easily. The valves though competent when tested with water, were very large and thin. The right ventricle also showed signs of dilatation. From their $\frac{HW}{BW}$ ratios it is apparent that all of the dogs in this litter had hypertrophied hearts.

Lungs. The lung weights of runners average within 2 per cent of the controls whereas the swimmers' lungs average 27 per cent smaller. Thörner (1930) reports significantly larger lungs in his dogs exercised by running.

Liver. The $\frac{\text{liver}}{BW}$ ratio of runners shows no important deviation from that of the controls while that of the swimmers exceeds it by 16 per cent. This high percentage is no doubt to be associated with the fact that the swimmer 47H, whose liver shows the greatest divergence from the control, was inactive for a number of weeks before autopsy. It is not difficult to postulate a large fuel storage in a dog whose nutritional level had been raised by a training period. Külbs (1906) and Hatai (1915) report larger livers in exercised animals whereas Thörner's exercised dogs had smaller livers than the controls.

Kidneys. Whereas the kidneys of our runners are only 3 per cent in excess of the controls, those of the swimmers are 26.7 per cent larger. Hatai (1915) reported the kidneys of exercised rats to be 20 per cent enlarged, and Thörner's (1930) exercised dogs showed an increase of 4 per cent. Borovansky (1930) also reports an increase due to exercise.

Spleen. Our runners show a 6 per cent spleen enlargement whereas the swimmers show a 20 per cent reduction. Hatai found a decrease of 20 per cent in exercised rats while Thörner found no change.

Thyroid. In the runners our data show a 20 per cent increase in this organ while in swimmers the increase is 34 per cent. Hatai reports a decrease of 13.44 per cent in exercised rats.

Adrenals. Our runners show a 21 per cent reduction in adrenal weights whereas the swimmers show an increase of 28 per cent. Borovansky (1930) reports slight evidence of adrenal enlargement resulting from exercise while Hatai (1915) reports no change in the male and 47.76 per cent increase in the exercised female rat.

Testes. The runners in our series show 9 per cent smaller and the swimmers 38 per cent smaller testes. Hatai (1915) reports increases of 12 per cent in exercised rats.

Bone marrow. In his 1912 paper Külbs reports having observed a striking difference in the bone marrow of exercised dogs compared with controls. Whereas the marrow of unexercised dogs was yellow and fat, in his exercised dogs it was red. Külbs reproduces a picture in which this difference may be detected by a difference in the shade of gray representing the respective bone marrows. We have examined our material with his findings in mind. Though we have had an artist paint a number of these specimens we have found no way of reproducing them satisfactorily. A brief description will, therefore, have to serve. In litter B the leg bone of the runner showed a decidedly deeper shade of red. In litter D there was no detectable difference. In litter E the marrow of the runner was palest, that of the swimmer was reddest and that of the control intermediate. In litter H the runner and swimmer both showed markedly redder bone marrow than did the control.[1] Broun (1923) has reported similar observations and correlates this finding with his position that exercise stimulates the marrow to increased erythrocyte production.

Differences between swimming and runnning exercise. In view of the rather marked differences observed in the weights of certain organs from dogs exercised by running as compared with swimming, it is in order that we point out the differences in these two forms of exercise as employed by us.

1. Running is voluntary and unburdened whereas swimming is forced and the animal is loaded with weights about the shoulders.

2. Respiration is rapid and extensive in running while in swimming it is slow and labored.

[1] Since this was written one of us discussed these findings with Professor Külbs at Cologne and learned that this worker had in later studies also observed exceptions in which exercised animals showed paler bone marrow. He is inclined to seek the explanation of these exceptions in impaired nutritional states arising quite independent of the exercise program.

3. The overventilation in running tends to induce an alkaline blood picture with low CO_2 content whereas in swimming there is a tendency toward acidosis and higher CO_2 tensions (Rice and Steinhaus, 1931).

4. While running there is a greater increase in body temperature than in swimming in water at 25°C.

5. It is possible that in running there is less rise in blood pressure due to the greater vasodilatation attending the higher body temperatures and lower CO_2 tensions.

Although our data are entirely inadequate to establish conclusions concerning the different organ stimulating effects of running and swimming exercises, it may not be amiss to point out that the kidneys, spleen, and adrenals which seem to be affected most differently by running and swimming are all particularly sensitive to conditions of asphyxia with its attendant increases in CO_2 tension and blood pressure. There may also be some relation between the markedly smaller lung weights and the limitations to respiratory movements imposed by swimming.

SUMMARY

1. A total of eleven dogs of which four were held as unexercised controls while seven were exercised by running or swimming, were autopsied and organ weights compared.

2. Exercise induces a true work hypertrophy in the heart which under the conditions of our experiment was more marked in swimmers than in runners.

3. The cardiac hypertrophy involves both ventricles with just a little excess in favor of the left heart.

4. There is no indication of a comparable hypertrophy of the skeletal musculature of the limbs nor of the limb bones.

5. Lungs, liver, kidney and spleen show no significant changes in dogs exercised by running, whereas the adrenals and testes show significant reductions and the thyroid shows marked enlargement.

6. In dogs exercised by swimming the lungs, spleen, and testes show significant reductions whereas the kidneys, thyroid and adrenals show marked enlargement and liver changes are uncertain.

7. Though it is recognized that the body of data is small, the differences in the response of the lungs, kidneys, spleen and adrenals to the two forms of exercise used in this study are so large and unmistakable that they cannot go unnoticed. The two forms of exercise are compared with special reference to these differences.

8. In four of the seven exercised dogs the bone marrow was markedly redder than in their controls, in two there was no difference while in one the marrow was actually paler than in the control. These differences are not related to the mode of exercise.

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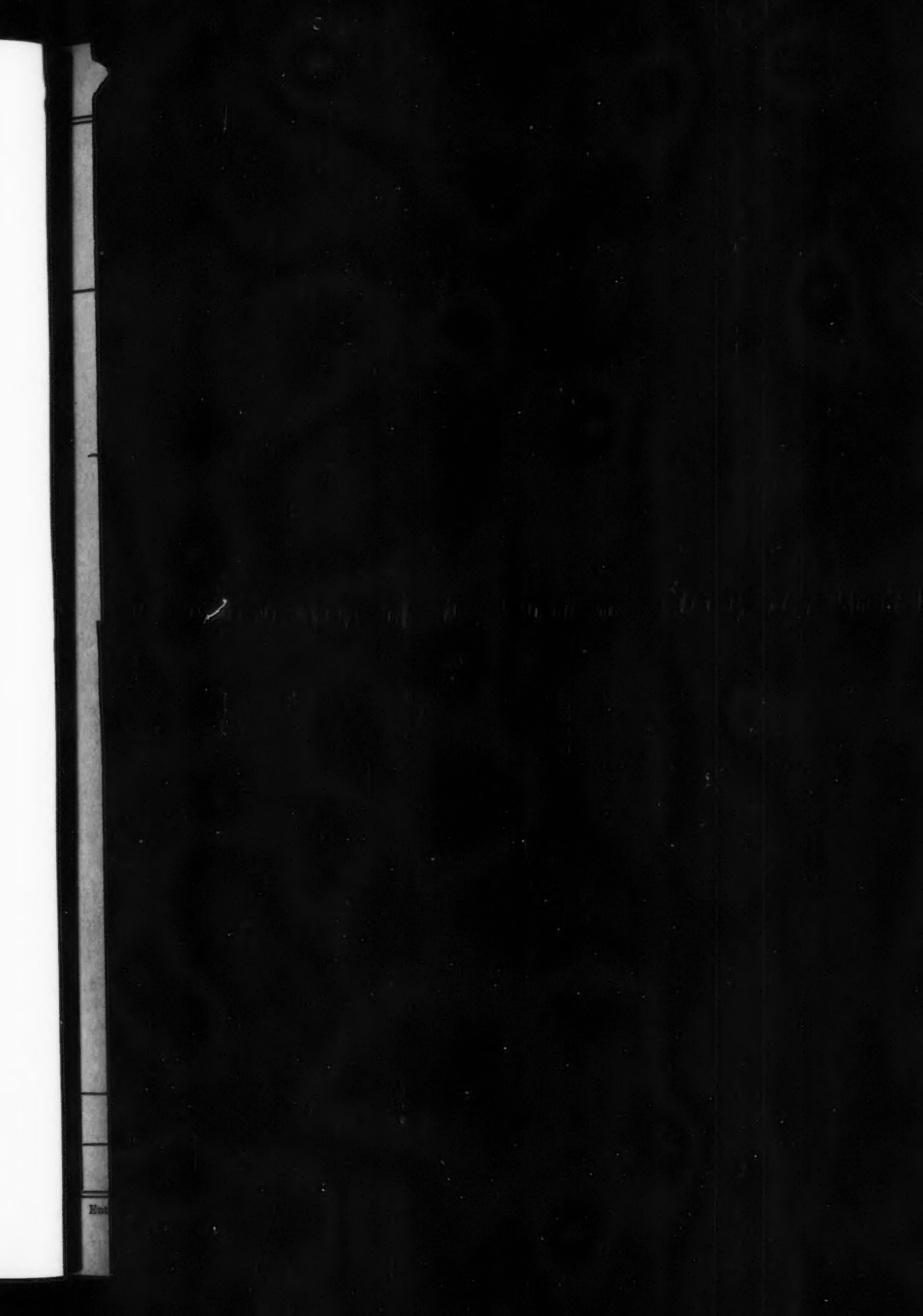
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